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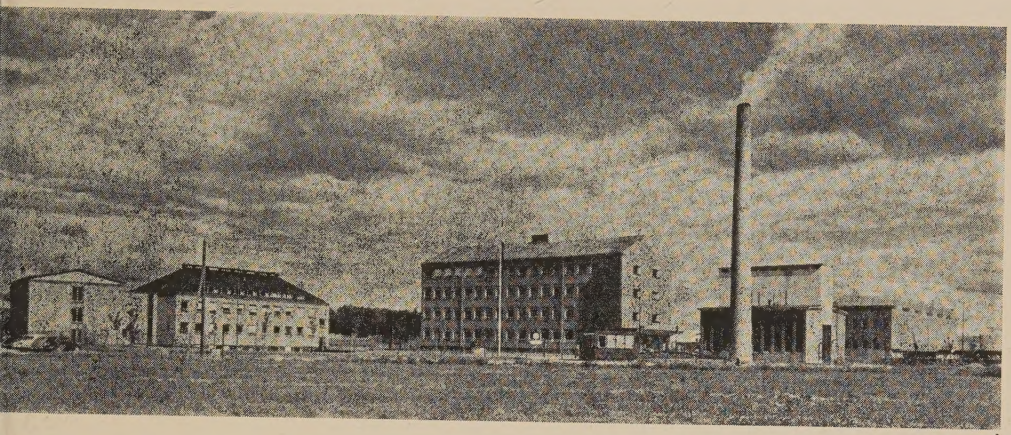

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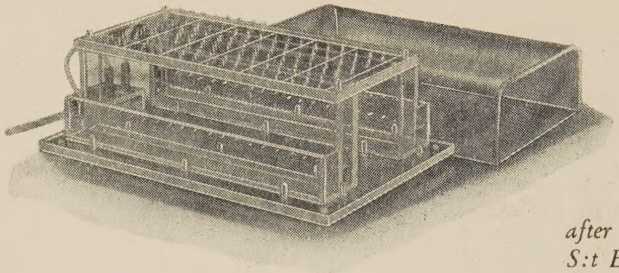

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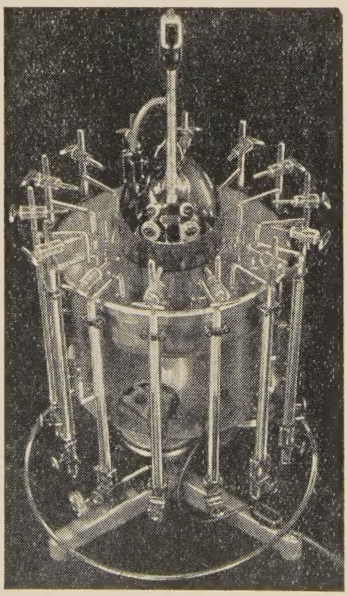

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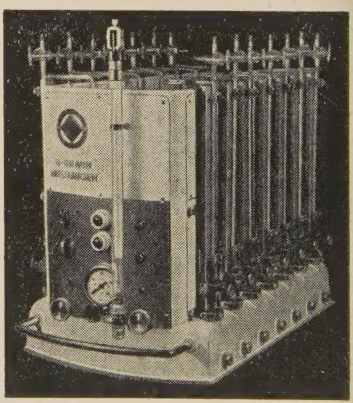

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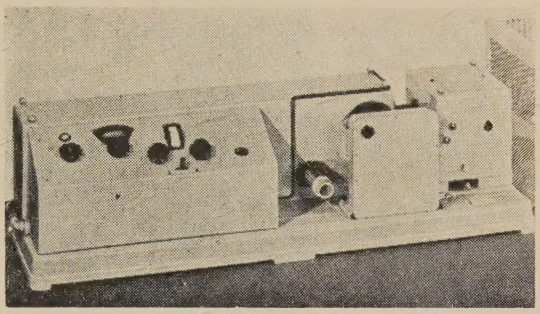

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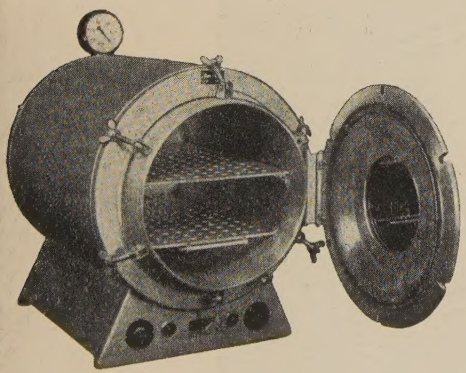

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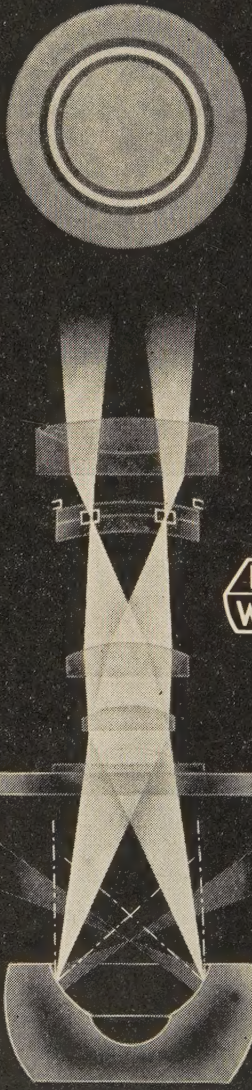

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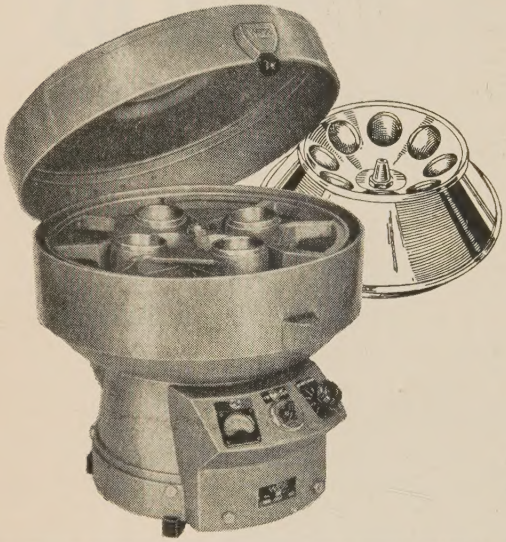

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5856-100

STUDIES ON HEPARIN AND THE LIPEMIA CLEARING FACTOR. I.

By B. HOOD, G. ANGERWALL, B. ISAKSSON and G. WELIN

From the First Medical Department, Sahlgren's Hospital and Institute of Medical Chemistry, Medical School, Gothenburg, Sweden

(Received for publication Aug. 21., 1953)

Anfinsen, Boyle & Brown (1952) have monstrated the presence of a number of nponents in the clearing of lipemic plasma ociated with heparin.

Graham, Lyon, Gofman, Jones, Yankley, nonton & White (1951) showed that parin caused a profound shift in the lipo-teins of plasma from those of higher S es to those of lower. The remarkable iciency of heparin in this conversion of oproteins led these authors to assume that accumulation of abnormal amounts of 12—100 found in myocardial infarction, ential hypercholesterolemia, etc., might due to a deficiency of a heparin-like sub-nce.

Block, Barker & Mann (1951) found with dose of 3 mg of heparin less clearing of mentary lipemia in the majority of athero-erotic individuals than in normals.

The remarkable efficiency of heparin in anging the pattern of plasma lipoproteins uld suggest that the abnormal accumulation of lipoproteins of S_f 12—100 might be due to a deficiency of heparin. On the other hand, the different response in clearing to identical doses of heparin in the Block, Barker & Mann series could be interpreted in various other ways. One might assume a lack of other components making up the complete clearing factor or a presence in the atherosclerotic individual of factors with an inhibitary influence on the clearing.

We decided to attempt a further analysis of these questions.

During the course of this work a few observations, not directly related to this main question, are worth noting.

METHODS

Venous oxalated blood samples were centrifuged at 2000 r.p.m. for 20 minutes as soon as possible after withdrawal. Lipemic plasma was obtained 3 hours after a standard fat meal, approximately 1.5 g fat/kg body weight (butter and cream). Blanks of lipemic plasma, lipemic plasma + preheparin plasma, lipemic plasma + NaCl and

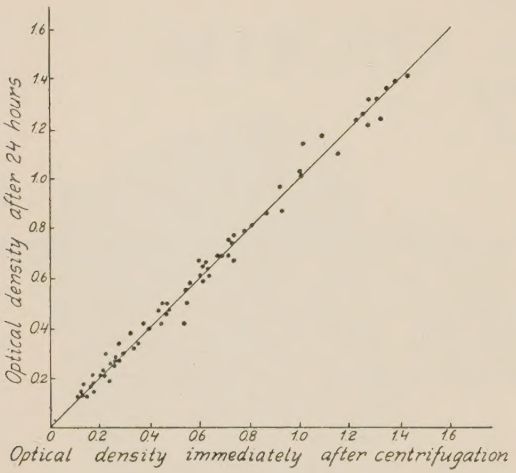

Fig. 1. Optical density of lipemic plasma at 650 mμ —10 mm path. Room temperature.

usually postheparin plasma were run in every series together with the tests. The readings were made in a Beckman spectrophotometer model DU or B at 650 mμ in 10 mm Corex cells. The optical density is markedly influenced by temperature. Therefore, care was taken to bring cold stored plasmas to room temperature before entering them in any experimental series. Lipemic plasma showed a surprising constancy of the optical density in closed tubes at room temperature for periods of up to two or three days. In Fig. 1 this is borne out for the first 24 hours.

RESULTS

When does optimal clearing activity of plasma occur after injection of heparin?

Blodo samples were drawn from normals before injection of 100 mg of heparin and 10, 20, 30, 60, 90, 120, 150, and 180 minutes respectively after the injection of heparin. After centrifugation these plasmas were mixed with lipemic plasmas from two subjects, in both series in duplicates. There was a steep rise in clearing activity during the first 30 minutes after injection, a fairly constant level between 30 and 90 minutes and a slow decline from 90 to 180 minutes (Fig. 2). For further work we decided to use a plasma collected 60 minutes after intravenous injection of 100 mg of heparin. This we denoted as our active standard plasma —$P_{Hep\ 100-60}$.

Blockade of clearing activity due to protamine sulphate. Anfinsen, Boyle & Brown found their most clearing-active Cohn fraction to contain heparin far in excess of that predicted by uniform distribution of the injected material in the total plasma space. Assuming that the strong negative charge of heparin might be involved in the clearing reaction, we tried another substance with a large molecule and a negative charge as

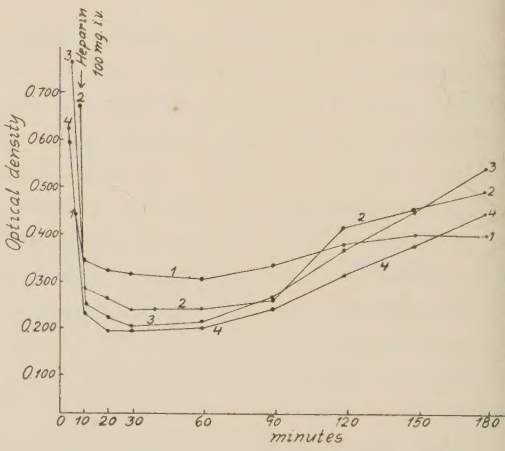

Fig. 2. Lipemic clearing activity of plasma after injection of 100 mg heparin (3 postheparin; 6 lipemic test. pl.). Control before heparin injection 2 cc preheparin + 2 cc lipemic pl. Postheparin pl. 2 cc + lipemic pl. 2 cc.

Curve shows clearing 24 hours after mixing; 23 hours + 4°, 1 hour + 37°.

ıg as that of heparin.[1] This produced race of clearing activity in the same time. n the other hand, protamine sulphate found to exert a blocking activity in the ing process in proportion to the concen- on used — within the range of 0.025— 025 per cent. This occurred whether amine sulphate was present from the et or was added when the clearing reac- was developing rapidly (Fig. 3). Com- nts of protamine, the basic diamino , *i. e.* arginine and histidine, showed no of blocking activity. Hexamethonium es a very strong positive charge and has all molecule but no trace of blocking ity was found even in high concentra-

ATERIAL FOR CLINICAL STUDY

y endogenous factors of importance in osclerosis should be predominantly at in cases exhibiting manifest athero- sis at a comparatively young age. We therefore chosen as representative for therosclerosis group, one group of sub- below the age of 50 years, who had ardial infarction, and one group of cts with essential hypercholesterolemia ascertained vascular damage. (In a s of 12 xanthomatous subjects Gofman associates found very high rates of S 100.) As controls we have used young iduals below the age of 35 years, clinic- ealthy (physicians, nurses) or suffering peptic ulcer or lumbago.

e have raised the following questions:

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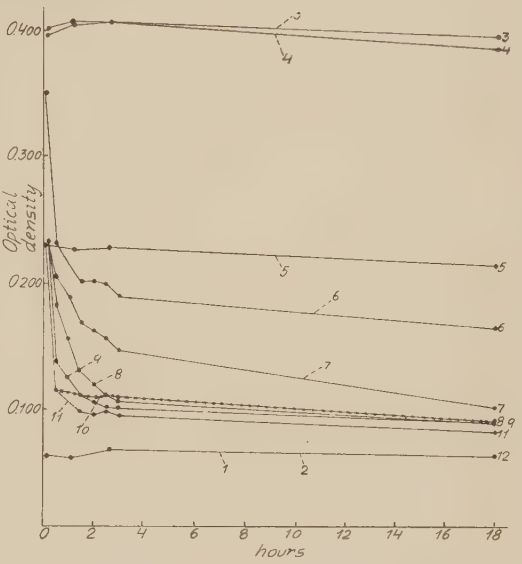

Fig. 3. Blocking effect of protamine sulphate on clearing of lipemic plahma treated with postheparin plasma (22° C).

1. Posthep. plasma.
2. Posthep. plasma + 1:40 0.2 per cent prot. sulph.
3. Lipemic plasma
4. Lipemic plasma + 1:40 0.2 per cent prot. sulph.
5. Preheparin pl. + lip. pl. + 1:40 0.2 per cent prot. sulph.
6. Postheparin pl. + lip. pl. + 1:40 1 per cent prot. sulph.
7. Postheparin pl. + lip. pl. + 1:40 0.2 per cent prot. sulph.
8. Postheparin pl. + lip. pl. + 1:40 0.05 per cent prot. sulph.
9. Postheparin pl. + lip. pl. + 1:40 0.02 per cent prot. sulph.
10. Postheparin pl. + lip. pl. + 1:40 0.2 per cent prot. sulph added after 30 min.
11. Postheparin pl. + lip. pl.

1. Is alimentary lipemic plasma from the atherosclerotic cleared *in vitro* at the same rate as lipemic plasma from the normal indi-

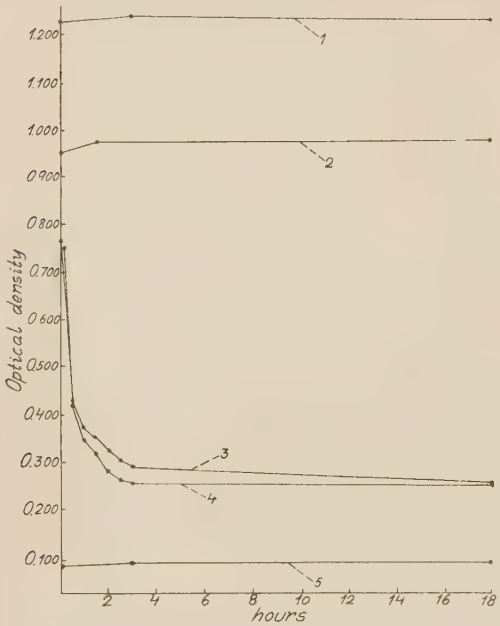

Fig. 4. Lipemic clearing activity of plasma after injection of 100 mg heparin (22° C)

Essential hypercholesterolemia — 5 cases.

1. Patient's lipemic pl.
2. Patient's lipemic pl. + patient's preheparin pl.
3. » » » + patient's postheparin pl.
4. » » » + control. postheparin.
5. » postheparin pl.

vidual by the same clearing active plasma —$P_{Hep\ 100-60}$?

2. Does the atherosclerotic individual produce a $P_{Hep\ 100-60}$ as active as a normal one?

3. Is the clearing rate normal in a system of $P_{Hep\ 100-60}$ and lipemic plasma from the same atherosclerotic subject, *i. e.* in the absence of any plasma from normals?

Figures 4 and 5 show the clearing rates of lipemic plasma from atherosclerotic individuals when run against postheparin plasma from the same subjects as compared wi postheparin plasma from young norma The other alternatives give essentially t same picture.

If $P_{Hep\ 100-60}$ is moderately diluted (t to 25 times) the lower clearing velocity w be a more sensitive indicator of the conce tration of clearing factor present. (The studies will be published in another conne tion.) In four experiments $P_{Hep\ 100-60}$ fro cases of essential hypercholesterolemia a normals has been diluted 3:10, 2:10, 1:1

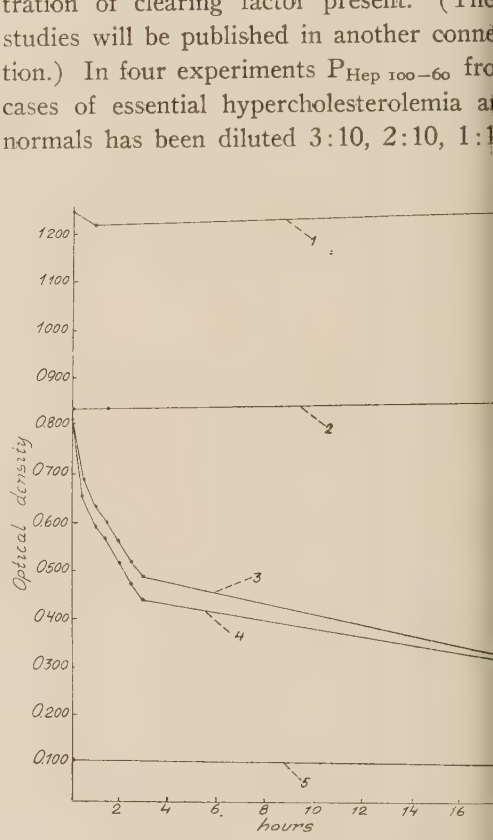

Fig. 5. Lipemic clearing activity of plasma af injection of 100 mg heparin. (22° C).

Myocardial infarction — 6 cases.

1. Patient's lipemic pl.
2. » » » + patient's preheparin
3. » » » + patient's postheparin
4. » » » + control. postheparin
5. » postheparin pl.

1:20 and run against lipemic plasma the same subjects. No significant difce in clearing rate was found. In both os there was, as seen in Fig. 4 and 5, the undiluted $P_{Hep\ 100-60}$ a slightly r clearing velocity for the controls. As did not appear in the dilution experis we have considered this difference to significant.

nclusively, no significant difference be- the normal and atherosclerotic indil has been found in the production of ing factor or in the clearing of aliary lipemic plasma. Thus, we have no evidence for a deficiency of the components necessary for the producf the complete clearing factor. Further-, in one xanthomatous subject there was three weeks' injections of 100 mg of rin three times a day, at least the same ity one hour after the first injection in norning as in a control $P_{Hep\ 100-60}$. If k of the lipoprotein conversion factor is cause of the abnormal accumulation of roteins of S_f 12—100 described in rosclerosis, there seems to be reason to me a deficiency in heparin itself.

he problem of spontaneous clearing ity. A main aim has been to investigate her spontaneous clearing activity occurs, without administration of heparin.

a series of six subjects, oxalated blood drawn 3 and 5 hours respectively after andard fat meal 1.5 g fat/kg body ht). On cooling the blood from body om temperature there is a definite ine in optical density. As we suspected this might interfere with any slight

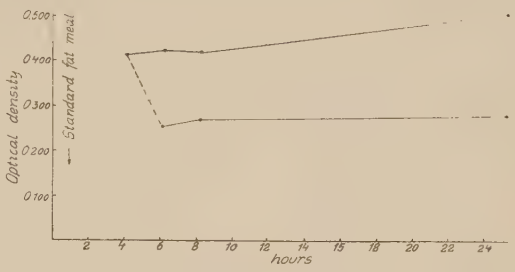

Fig. 6. Clearing of alimentary lipemic in vivo. Absence of clearing in vitro at 37° C (6 normals).
1. Plasma collected 3 hours after standard fat meal, continuously kept at 37° C.
2. Plasma collected 5 hours after standard fat meal, continuously kept at 37° C.

clearing which might occur, the samples wer drawn into thermos bottles of 37°, kept and centrifuged in a couveuse at 37° and read in warmed cells. One duplicate series was kept and read at room temperature. As is seen from Fig. 6, no clearing occurred in plasma continuously kept at 37° *in vitro,* while a very considerable decrease in the density of the lipemic plasma occurred *in vivo* between the third and the fifth hour after the fat meal. Nor did the lipemic plasma clear when kept at room temperature.

Fasting plasma incubated with lipemic plasma for 24 hours at room temperature showed no tendency to clearing. Thus, we have not been able to trace spontaneous clearing activity in venous plasma with these techniques, either in the fasting state or in the clearing phase after a fat meal.

Preliminary attempts to concentrate the clearing factor. According to Anfinsen, Boyle & Brown 1952, clearing factor is concentrated mostly in Cohn fraction III, 1,2,3.

Prothrombin also appears in this fraction. We decided to study whether the clearing factor followed prothrombin in other aspects. Owren (1946) showed that prothrombin was not absorbed on 20 per cent asbestos filter pads, but practically completely on 50 per cent asbestos. There was no measurable loss of clearing activity when filtering active plasma through the 20 per cent asbestos but complete loss after filtering through 50 per cent asbestos in the three experiments performed. We have, however, as yet failed to eluate the clearing factor from the asbestos.

Adopting Seegers' method for isolation of prothrombin on active standard plasma —$P_{Hep\ 100-60}$ and testing fractions from all steps for clearing activity, a considerable activity was found in step 3 (the acetic acid precipitate of diluted plasma dissolved in a weakly alkaline solution). In all the following steps no trace of activity was found, possibly indicating that the factor was absorbed on the $Mg(OH)_2$ but not eluated with the carbon dioxide. Further tests showed complete loss of clearing activity after shaking P_{Hep} of varying concentrations with $Mg(OH)_2$.

SUMMARY AND CONCLUSIONS

1. The optical density of alimentary lipemic plasma at 650 mμ is essentially constant at room temperature for 24 hours.

2. The optimal clearing activity is reached within 30 minutes after a large injection of heparin (100 mg i.v.). There is a plateau of optimal activity between 30 and 90 minutes after the injection and then a slow decline.

3. Protamine exerts a strong inhibitio on the clearing process proportionate to th concentration used. This is considered t indicate that the negative groups of hepari play a rôle in the effect of the clearing facto Small molecules with basic groups, *i.* arginine, histidine, hexamethonium chlorid exert no inhibitory influence. A large mol cule with strong negative charge (polyflore tinphosphate) did not elicit clearing activit on injection.

4. The ability to produce clearing facto after injection of 100 mg of heparin wa entirely similar in atherosclerotic (myo cardial infarction and essential hypercholo sterolemia) and normal individuals. Th also applied to the tendency of the alimentar lipemia to clear on addition of the sam active plasma. The clearing occurred at th same rate when lipemic plasma from th atherosclerotic individual was incubated wit postheparin-plasma from the same individua *i. e.* in the absence of any "normal" plasm

5. Spontaneous clearing activity has bee found neither in fasting plasma nor in lipem plasma drawn in the clearing phase aft a fat meal.

6. Some possible ways to concentrate th clearing factor following the methods fo preparation of prothrombin have been ou lined. Both the factor and prothrombi appear in the Cohn fraction III—1, 2, according to other workers, both are com pletely absorbed on 50 per cent asbestos an on $Mg(OH)_2$ and both are precipitated wit acetic acid at pH in diluted plasma.

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DOES INTERMITTENCY OF GLOMERULAR ACTIVITY OCCUR IN HUMANS?

RENAL FUNCTIONAL STUDIES IN PNEUMONIA

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In his W. H. Welch Lectures on the kidney, Homer Smith (1943 a) mentions the abnormally high urea clearance values one may see in febrile conditions, for instance in pneumonia. Only a few papers have treated this interesting problem, and only a very few of them have adapted a uniform experimental procedure which makes a proper interpretation possible.

Although reports to the contrary have appeared, most papers agree that in the acute stage of lobar pneumonia urea clearance may be increased, sometimes to abnormally high figures, up to 300 per cent or more according to Goldring (1931). This may especially be true in patients less than 40 years of age (Farr & Abernethy 1937). Following the crisis a decrease in urea clearance has been described, sometimes to abnormally low figures (Goldring 1931). A return to normal figures usually occurs within a few days or weeks (Goldring 1931, Lewis 1917, McIntosh & Reiman 1926). A depression of longer duration has also been reported (Farr & Abernethy 1937). The relation between urea clearance and other clearances has been examined only with regard to the excretion of phenol red, but as these investigations suffer from the failure of taking the blood concentration of phenol red into consideration, we do not think them relevant for d cussion.

It is therefore unknown whether the i crease in urea clearance is a limited fu tional change, or part of a change on a mo general scale. The problem is of interest it throws light upon fundamental princip in renal physiology, which will be discuss below.

The scope of the present paper is examine the renal function in lobar pne monia, making use of urea clearance, end genous "creatinine" clearance, inulin cle ance and para-aminohippuric acid clearan in order to obtain information about glom rular and tubular function and renal plas flow. When we first planned this study, knew beforehand that we could expect o a few patients to show increased urea cle ance values, as a patient with lobar pne monia often is very ill, and frequently ev suffering from shock. It is known that th conditions will favour a depression of re function. This is in our opinion the reas why some authors have found normal even low values for urea clearance duri the acute stage of lobar pneumonia. At time when most papers dealing with t problem were published, knowledge of re function during shock or acute illness w lacking.

MATERIAL AND METHODS

e examined 32 patients, all of whom ally and roentgenologically suffered lobar pneumonia. As mentioned above, ill patients will show very low clearance s. One patient who was very exhausted had lowered blood pressure, had urea ance of 5 ml/minute and endogenous tinine" clearance of 37 ml/minute. After day's treatment urea clearance had to 35 ml/minute and endogenous "crea-e" clearance to 102 ml/minute. There no signs of renal disease. Experiences this made us try to pick out patients were not suffering from exhaustion or k. Nevertheless only 9 of a total of atients showed the response we were ng for, i. e. the high clearance response eumonia. We made sure that none had treated with sulfa preparations or peni- beforehand, as sulfa gives the same ical reaction as para-aminohippurate, penicillin can change the excretion of -aminohippurate.

ie patients were examined shortly after ttance to the hospital. One half to one before the examination they were given iter of water to increase diuresis. When urea and endogenous "creatinine" clear- s were performed, the tests were run two hours. In all cases catheterizations performed. In the instances where also n and para-aminohippuric acid clear- s were studied, the usual procedure was (priming and sustaining infusions, ler irrigation with water and injection r, etc.). In these cases two clearance ds were employed, and the figures sent averages of the two periods. In some cases the study was repeated after the temperature had become normal.

Urea was determined by Van Slyke's manometric method, in some cases controlled by the Conway method.

Endogenous "creatinine" was determined by the Jaffé reaction (saturated picric acid solution and 10 per cent NaOH).

Inulin was determined by the diphenylamine method as described by Corcoran & Page (1939) and Alving, Rubin & Miller (1939).

Para-aminohippuric acid (PAH) was determined by the method given by Homer Smith *et al.* (1945) with the exception that plasma protein was precipitated by trichloracetic acid instead of cadmium sulphate.

RESULTS

As mentioned in the preceding chapter, we found changes in the renal function of the type increased clearance values in only 9 out of 32 patients. In the other 23 cases the clearance values were either normal or slightly lowered. For reasons already given, only cases showing the high clearance type are of interest in this study. The results from the 9 cases showing this type are listed in the table.

It will be seen that in patients suffering from lobar pneumonia, the urea clearance may be considerably increased. In one instance a value of 140 ml/minute was found, *i. e.* nearly 200 per cent of the normal average. The values are on the whole elevated, especially when one considers that they have been obtained in patients suffering from a serious infectious disease which, because of circulatory changes, has a strong tendency to lower the clearance values. This fact gives even normal clearance values a special significance in pneumonia.

Table I. *Renal function in 9 patients in the acute stage and during convalescence of lobar pneumonia.*

Subjects	Age (years)	Date	Urine flow (ml/min.)	Clearances (ml/min.)			
				Urea	End.cr.	Inulin	*PAH*
I. A. ♀	51	19/5 49	0.9	91	100		
		27/5 49	4.6	47	91		
M. S. ♀	17	18/6 49	5.8	122	268		
		24/6 49	1.3	88	119		
A. T. ♂	49	16/1 51	7.2	140	231		
		22/1 51	4.2	90	155		
		2/2 51	4.5	45	100		
B. N. ♂	43	19/1 51	1.4	73	237		
		26/1 51	4.8	86	157		
A. T. ♂	24	15/1 51	2.1	116	187		
		22/1 51	8.0	106	156		
		24/1 51	1.3	88	121		
L. K. ♀	37	13/3 51	2.7	94	136		
		31/3 51	5.2	53	74		
K. S. ♂	45	30/3 51	6.0	119	122	254	1326
		10/4 51	4.2	69	95		
H. J. ♂	39	17/9 51	1.5	72	110	144	
T. S. ♂	26	5/12 51	5.0	103	148	187	1006
		14/12 51	5.5	81	132		

In the patient mentioned where urea clearance was 140 ml/minute, the endogenous "creatinine" clearance was 231 ml/minute, *i. e.* a little more than 200 per cent of the normal mean. In another case an inulin clearance of 254 ml/minute was found, *i. e.* more than 200 per cent of the normal mean. In this case PAH clearance was 1326 ml/minute, and this is also about 200 per cent of the normal average.

As all patient in this study were completely free from signs of renal disease, endogenous "creatinine" clearance can be taken as a sufficiently exact measure of the glomerular filtration rate.

The table shows that all clearances are changed in the same direction and mostly on the same scale, making their ratios normal.

When the examinations were repeated after the temperature had become normal, the clearance values had usually return to normal levels. No very low values we found during convalescence in this study.

DISCUSSION

Our results confirm previous findings th in lobar pneumonia urea clearance may som times be increased up to figures rarely never seen normally. In addition we ha found a corresponding increase in end genous "creatinine" clearance, and in so few instances where inulin and PAH clea ances have been examined, also these valu were correspondingly increased. In spite some very great increases in these valu filtration fraction and the ratios urea clea ance/inulin clearance and urea clearan endogenous "creatinine" clearance rema unchanged.

is shows that the increased urea clear-
is due to increased glomerular filtration
nd not to a decreased tubular reabsorp-
or back diffusion of urea.

the convalescent period a decrease in
e values mentioned was found, and the
ase was of the same magnitude for all
s. In our cases no decrease to abnor-
low values was found, but this might
e to the timing of the examination.

r findings of high clearance values
not constant. As mentioned above one
nly rarely expect to find a patient who,
suffering from pneumonia, exhibits no
of circulatory failure.

think that the changes in renal func-
lescribed above depend on "normal" or
npered renal response to the disease,
sponse which is abolished by serious
ons like bodily exhaustion. In some
nces the two responses, the increase and
lecrease, must be assumed to balance
y, resulting in normal clearance values
ding to the Van Slyke standard.

have examined only patients suffering
lobar pneumonia with the diagnosis con-
d by X-ray. There is, however, no doubt
our findings correspond to the observa-
regarding urea clearance in erysipelas
ther febrile diseases reported in earlier
ture. The increase in clearance values
g the febrile stage to abnormally high
, and the decrease sometimes to ab-
ally low values during convalescence,
n all probability a feature common to
al diseases.

has been stated in the literature that
ever itself is not responsible for the in-
e in urea clearance (Farr & Abernethy 1937). However, we deem it futile to try to correlate fever and renal functional changes because, as mentioned above, at least two conflicting responses are active in these patients, one leading to an increased and one to a decreased function. It is impossible to ascertain who is well enough for exhibiting the high clearance response and who is not. One highly febrile patient may show in-increased clearances for reasons so far unknown, and another highly febrile patient may show low clearances because of exhaustion or circulatory failure. The last type of reaction is probably the more common.

Concerning the mechanism of the high clearance type, it is important that artificial fever does not lead to increased glomerular filtration rate (Homer Smith 1943 b) or increased urea clearance (Farr & Moen 1939) although renal blood flow is increased (Homer Smith 1943 b). This observation has been used by Homer Smith as an argument against glomerular intermittency.[1] According to him the renal response to artificial fever and certain drugs consists only in changes in renal blood flow with no or only slight changes in glomerular and tubular activity. In cases of glomerular intermittency another type of response ought to be observed, that is an all-over even change in

[1] Richards & Schmidt (1924) when making glomerular and tubular punctures in the frog observed that not all glomeruli were open at the same time, but some opened as others were closing. This function, which is called intermittency of the glomerular activity, has later been confirmed in other cold-blooded animals. It has been questioned or denied in humans, especially by Homer Smith (1943 c).

blood flow and glomerular and tubular function.

But this is exactly what takes place in some patients suffering from lobar pneumonia as shown by us.

This brings up the question whether the all-over changes in renal function in pneumonia may be due to the opening up of a greater percentage of glomeruli in the acute stage, and a closing down later on.

As far as we can see, the arguments against intermittency are very conflicting. Some of the more important of them have been seriously questioned, among others by Kruhøffer (1950). A detailed discussion of the problem will be found in his thesis, as well as in Homer Smith's lectures. We don't feel that the arguments against intermittency based on the mathematical interpretation of the maximal glucose reabsorption curves (Homer Smith c) are convincing. A regulatory mechanism leading to an opening up of an almost equal number of glomeruli as those closing down, ought to give about the same average function as the same number of constantly open glomeruli. No mathematics, however ingeniously employed, can press more information out of simple averages, such as the clearance values, than sound reason. A regulatory mechanism such as sketched here would be no more delicate than many others known to physiology. The existence of glomerular intermittency would lend to the renal capillaries the same functional ability that we know since Krogh's "Anatomie und Physiologic der Capillaren" to be a general capillary characteristic. Such a function would readily explain how renal clearances vary as much as they do. Even the "glomerular activity" of Homer Sm varies between 60 and 150 per cent of average.

The really great changes in renal funct as seen in febrile diseases are not usua found except in these conditions. But think that changes corresponding to th are seen when one and a half kidney removed surgically. From the studies Kolberg & Høeg (1953) on normal d we know that the resulting function of remaining half kidney corresponds to ab 70 per cent of the normal averages, all cle ances mentioned in this paper being eve reduced. This corresponds well with results obtained by Kolberg, Høeg & Se (to be published) in humans with re tuberculosis. This means that the remaini portion has a function that corresponds 280 per cent of normal function. As no n glomeruli are formed in these conditio we feel it must be very difficult to expl these great increases in function only throu dilatation of glomeruli already dilated befo hand. Such a mechanism can hardly expl the constancy of the functional results of removal of three-fourths of the kidney s stance.

If intermittency really exists, it must admitted that larger changes in the perc tage of functioning nephrons take place o under special conditions like febrile disea and extreme reductions of kidney tissue in surgery and probably also when destr tion of kidney tissue is caused by patholog conditions like tuberculosis.

However, many present views on re function would have to be changed. T

"maximal" for certain reabsorptive excretory tests now in use would have e dropped. So would conclusions based hem.

may be added that Perez-Tamayo and nandez-Peon (1953), by injections of a-ink on cats have shown that intermit-y may take place in mammals on the ra-glomerular level". They state that the d does not circulate simultaneously ugh all the capillary loops within the erulus and that although there is no dence in the mammal for glomerular mittency in the sense of completely ive glomeruli, there are indications of mittency at an intraglomerular level". brings up the possibility that the may happen in humans, and that the ges in renal function discussed above be due to intermittency on the intra-erular level.

he significance of supernormal clearances ebrile diseases is of course independent hether they are due to glomerular inter-ency or not. But we think that the onstration of these all-over and even ges in renal functions present evidence vor of intermittency. We also think that nly way of really proving the existence termittency would be direct observations he living kidney.

CONCLUSION

the acute stage of lobar pneumonia is, under favorable conditions, an even simultaneous increase in renal blood glomerular filtration and urea clear-. In the convalescent period the inverse ges take place. These changes may be due to intermittency of glomerular activity and present real evidence in favor of such a function in the human kidney. It is held that no real proof for or against intermittency is possible without direct observation on the living kidney.

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EOSINOPHIL CELLS IN FISH TAPEWORM (DIPHYLLOBOTHRIUM LATUM) CARRIERS

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osinophilia is often encountered in con-ion with various parasites and in many s is considered one of the symptoms of sitosis. In connection with an earlier stigation (Telkkä, Wahlström & Kou-es, 1951) the writers noticed how greatly information in the literature, including books, on the eosinophil cells of fish worm carriers varies. Bernth & Hagens 46) and French (1947) mention pro-nced eosinophilia as one of the symptoms sh tapeworm. Schulten (1943) and Faust 49) also state that the carriers of this site have more or less pronounced nophilia. Page, Turner & Wilson (1927) d, admittely in a fairly small material, fish tapeworm carriers have 4—17 per eosinophil cells. Infestation tests where man (Le Bas, 1924) and dog (Wardle, schall & Horder, 1937) were infested fish tapeworm have given varying lts ranging from an unchanged eosino-count to marked eosinophilia.

he authors' own investigation is con-ed with examination of the incidence of nophil cells in fish tapeworm carriers.

ATERIAL, METHOD AND RESULTS

he authors' material consists of 300 fish tape-n carriers and of 300 out-patient department nts as controls in whom no tapeworm eggs were found on microscopic examination of the faeces. Cases where a distinct allergic anamnesis or the use of medicines could be presumed to affect the number of eosinophil cells were eliminated from each group. Eosinophil cells were determined by means of Randolph's (1949) stain with propylene glycol. The determinations were made in the forenoon, before eating.

The results are given in Figs.1 and 2.

The results show that the count of eosinophil cells, absolute or percentual, does not differ in any way from the values of the control material.

DISCUSSION

Judging by the results of the present material the frequent statements in literature on the eosinophilia of fish tapeworm carriers do not hold and are probably based on in-

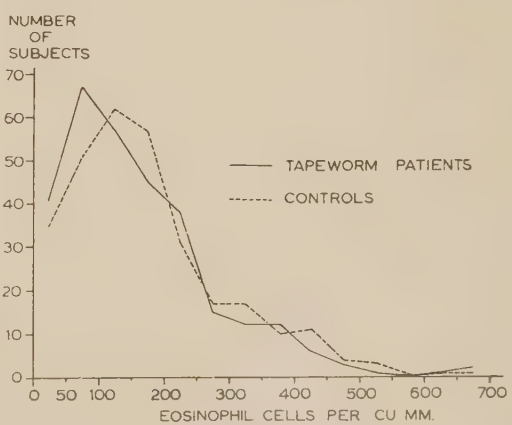

Fig. 1. Eosinophil cells per mm³ in tapeworm carriers and controls.

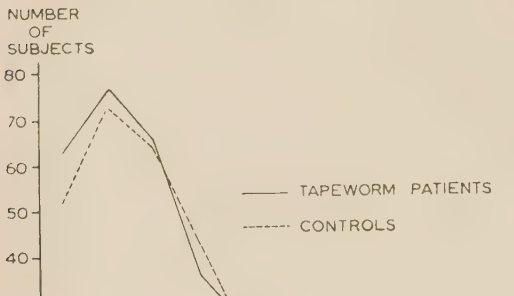

Fig. 2. Eosinophil cells per cent in tapeworm carriers and controls.

cidental individual cases and small materials. The present results are also supported by the values reported by Tötterman (1936) in his study of the eosinophil cells of the bone marrow and peripheral blood of tapeworm carriers. Bonnin & Moretti (1952) have published a review of eosinophilia caused by parasites and have ascertained it to be largely dependent on the species of parasite and its place of infestation. In their opinion parasites adhering to the inside of the intestine do not belong to those causing eosinophilia. The present investigation appears to confirm this supposition, at any rate for Diphyllobothrium latum.

SUMMARY

The investigation, which is based on the determination of eosinophil cells of 300 fish tapeworm (Diphyllobothrium latum) carriers and 300 control cases, leads to th conclusion that fish tapeworm does not caus eosinophilia, in spite of frequent statement to the contrary in the literature.

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GLUCOSE AND ACETONE AS SOURCES OF ERROR IN PLASMA »CREATININE« DETERMINATIONS

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two previous papers (Blegen & Haugen Haugen & Blegen 1953a) we stated that a "creatinine" values above 1.3 mg/100 ignify reduced renal function. We ed out, however, that in diabetics one find high plasma "creatinine" values o high blood sugar concentrations, as se reacts with alkaline picrate in the way as creatinine. In such cases we mended the use of a suitable brand oyd's reagent or addition of yeast to ve the glucose.

me examples will show the significance s procedure: A diabetic, 48 years old, n admission to the hospital blood sugar 0 mg/100 ml and plasma bicarbonate per cent by volume. Plasma "crea-" was 4.9 mg/100 ml, *i. e.* considerably ased. There was neither proteinuria nor astion or shock, and the high plasma tinine" value could not be explained by ssed renal function. The increased a "creatinine" value was thought to be o an increase of "pseudocreatinine", and roved to be true. The true plasma crea- value proved to be 0.82 mg/100 ml. treatment, plasma "creatinine" was ng/100 ml, true plasma creatinine 0.80 00 ml.

other diabetic, a woman aged 56 years, dmitted in precoma; blood sugar was 710 mg/100 ml and plasma bicarbonate less than 9 per cent by volume. The plasma "creatinine" value was 4.7 mg/100 ml. Undoubtedly she suffered from shock, but there was no proteinuria, and the high plasma "creatinine" value could not be explained by the shock alone. True plasma creatinine after shaking with Lloyd's reagent was 1.2 mg/100 ml. After addition of yeast and standing 4 hours at 37° C, blood sugar was 20 mg/100 ml and plasma "creatinine" 1.52 mg/100 ml. The Wishardt reaction on acetone in plasma, which before addition of yeast had been strongly positive, was negative. When the plasma bicarbonate and blood sugar after treatment had become normal, plasma "creatinine" was 1.48 mg/100 ml, true creatinine 0.81 mg/100 ml. It is of interest that the plasma filtrate, after boiling to remove acetone, showed a "creatinine" value of 2.98 mg/100 ml.

Evidently diabetics with high blood sugar may show increased amounts of "pseudocreatinine". It has long been known that glucose is a "pseudocreatinine". Gettler (1917) was of the opinion that also normal blood sugar values are of significance, the glucose giving only a slight augmentation when the values are read after 10 minutes, but "on standing longer than 10 minutes, the color developed by the glucose becomes

appreciable". He pointed out that it is necessary to read the color after 10 minutes, as "delay introduces an element of error".

Hunter & Campbell (1917) maintained that Gettler exaggerated the influence of glucose on the color development in the Jaffé reaction, admitted however that "quantities of glucose greater than normal react with considerably greater rapidity". They found that the addition of 0.4 g glucose to 100 ml of blood gave sufficient color within 10 minutes to raise the plasma "creatinine" value by —0.10—0.20 mg. On reading after 1 hour the increase would be 0.5 mg, and after 24 hours the color would be so dark as to be almost opaque. They concluded that "the glucose factor may assume considerable importance whenever there is notable hyperglycemia".

Other investigators, as well, are of the opinion that glucose really is a part of the "pseudocreatinine" (Lauson 1951, Haugen 1953).

There is, however, another factor in diabetics which may be of significance in the determinations of plasma "creatinine". In number two of the patients mentioned above boiling of the plasma filtrate led to a reduction of the "creatinine" value. This implies that acetone and perhaps other ketone bodies may be of importance. Bucht (1951) pointed out that the glassware used in colorimetric determinations of creatinine should not be washed with acetone, as this will increase the "creatinine" value.

The object of this study is to examine the influence of glucose and acetone on the determinations of creatinine in aqueous solutions and in plasma. With regard to the Jaffé reaction in urine it has been shown (Haug 1953) that dilutions of normal urine beha like aqueous solutions of creatinine.

METHODS

Known amounts of glucose and acetone in c centrations corresponding to those found in d betics were added to aqueous solutions of creatin and to normal plasma. Heparinized plasma v used, and the protein precipitated by the Wu (192 method. The "creatinine" was determined by Jaffé reaction (saturated picric acid and 10 cent NaOH). The colors were read after exac 10 and 40 minutes, temperature 23° C (the c bration curve was constructed on readings at same temperature).

True creatinine was determined by the aid Lloyd's reagent after the method described by H (1950) and slightly modified by Haugen & Bleg (1953 b). As the procedure requires some ti the colors were read after exactly 45 and 90 mi tes at 23° C.

A Coleman Junior spectrophotometer was us wave length 500 mμ.

RESULTS

Table I shows that the addition of 4 and 900 mg of glucose, respectively, to 1 ml of a known creatinine solution will ra the "creatinine" value significantly after minutes. Addition of 450 mg of glucose creases the "creatinine" level from 1 to 1

Table I. *The influence of glucose on to "creatinine" and true creatinine values*

	Total "creatinine" (mg/100 ml)		True "creatini (mg/100	
Reading time (minutes)	10	40	45	9
Aqueous solution of creatinine 1 mg/100 ml	1.00	1.00	1.00	1.0
+ glucose 450 mg/100 ml	1.14	1.30	1.01	1.0
+ glucose 900 mg/100 ml	1.30	1.55	1.02	1.0

ble II. *The influence of glucose on sma "creatinine" and true creatinine values.*

ng time (minutes)	Total "creatinine" (mg/100 ml) 10	40	True "creatinine" (mg/100 ml) 45	90
a	1.03	1.16	0.84	0.85
cose 350 mg/100 ml lasma	1.16	1.52	0.88	0.93
cose 700 mg/100 ml lasma	1.32	1.92	0.89	0.93

00 ml, and the addition of 900 mg of se will raise the "pseudocreatinine" to about the double level. When read 40 minutes the "creatinine" value will ase further, and the addition of 900 mg ucose will raise the value by more than r cent.

om the same table it will be seen that shaking with Lloyd's reagent the in- e is only slight. After 45 minutes 900 f glucose will raise the creatinine value nly 0.02 mg, and after 90 minutes the ase is only 0.05 mg/100 ml.

xactly the same result was found when se was added to plasma, which will be from Table II. When 700 mg of glucose

ble III. *The influence of acetone on "creatinine" and true creatinine values.*

ng time (minutes)	Total "creatinine" (mg/100 ml) 10	40	True "creatinine" (mg/100 ml) 45	90
us solution of atinine 1 mg/100 ml	1.00	1.00	1.00	1.00
etone 20 mg/100 ml	1.30	1.39	1.00	0.99
etone 40 mg/100 ml	1.70	1.90	1.03	1.05
etone 60 mg/100 ml	2.06	2.30	1.04	1.05

Table IV. *The influence of acetone on plasma "creatinine" and true creatinine values.*

Reading time (minutes)	Total "creatinine" (mg/100 ml) 10	40	True "creatinine" (mg/100 ml) 45	90
Plasma	1.10	1.26	0.96	0.95
+ acetone 20 mg/100 ml of plasma	1.35	1.60	0.97	0.97
+ acetone 40 mg/100 ml of plasma	1.66	1.95	1.03	1.05
+ acetone 60 mg/100 ml of plasma	1.94	2.26	1.04	1.06

was added to 100 ml of plasma, a considerable increase of the plasma "creatinine" value was found. After shaking with Lloyd's reagent the value was significantly lower (true creatinine). Here too the addition of glucose raised the (true) plasma creatinine reading, but the increase is very slight in proportion to the increase in the "total creatinine" value.

From Tables III and IV it will be seen that acetone has a considerable "creatinine simulating" effect. Addition of 60 mg of acetone to 100 ml of a known solution of creatinine will raise the "creatinine" value to more than double the value after 10 minutes, and after 40 minutes the increase is even higher. After shaking with Lloyd's reagent the increase is, however, only insignificant. Addition of 60 mg of acetone to 100 ml of a known solution of creatinine increases the creatinine value by only 0.04 mg, a little more after 90 minutes. In plasma the increase after shaking with Lloyd's reagent is a little higher, but also here insignificant in proportion to the increase in filtrates not treated this way.

Table V. *The influence of glucose + acetone on plasma "creatinine" and true creatinine values.*

	Total "creatinine" (mg/100 ml)		True "creatinine" (mg/100 ml)	
Reading time (minutes)	10	40	45	90
Plasma	1.13	1.30	0.92	0.92
+ glucose 600 mg/100 ml + acetone 40 mg/100 ml of plasma	1.88	2.98	0.98	1.02

When glucose and acetone were added simultaneously, similar results were found. Table V shows a considerable increase in plasma "creatinine" values; after shaking with Lloyd's reagent the increase is only slight.

DISCUSSION

It is shown that addition of glucose and acetone to aqueous solutions of creatinine will raise the "creatinine" values considerably, and the increase is dependent on the reading time. On addition of glucose and acetone to plasma the same results are found. It follows that these two substances belong to the "pseudocreatinines" and may represent considerable sources of error in determinations of plasma "creatinine".

The results confirm Hunter & Campbell's opinion that hyperglycemia may be of importance in the determination of plasma "creatinine". However, acetone may also give too high "creatinine" values. These substances influence plasma "creatinine" determinations in a way that may simulate renal failure.

The time-factor is of importance. As the maximal color of aqueous solutions of creatinine is developed after 10 minutes (Hunt & Campbell 1917, Haugen 1953), the colo ought to be read after this interval.

In normal individuals glucose and aceto will be of no importance for plasma "cre tinine" determinations. Hunter & Campb held the view that normal blood sugar valu would not raise plasma "creatinine" by mo than 0.03 mg/100 ml. This is confirmed the present study. In normals there are on insignificant amounts of ketone bodies. Ho ever, when the readings are delayed, bo glucose and acetone may be of importanc

It has been shown that nearly all of t "pseudocreatnine" given by glucose and ac tone may be removed by shaking wi Lloyd's reagent. After shaking only insign ficant amounts remain. Thus, 900 mg glucose added to 100 ml of aqueous soluti of creatinine will raise the "creatinine" val by only 0.02 mg, whereas 700 mg of gluco added to 100 ml of plasma will raise the lev by 0.05 mg. Much the same applies to ac tone. The reason why Lloyd's reagent do not remove all "pseudocreatinine" at ve high concentrations of glucose and aceto is not quite clear. Most probably some of t glucose or acetone will be left on the surfa of the Lloyd's reagent or on the inner si of the glasses in spite of the most caref handling. It will be seen from the tabl that the "pseudocreatinine" values are high at the reading after 90 minutes than aft 45 minutes, and this may signify that a re ding earlier than 45 minutes may give l "pseudocreatinine". It might be possible reduce the reading time to about 20 minut

The fact that Lloyd's reagent does n remove all "pseudocreatinine" is of mo

tical than practical significance, as the ty left is very slight. It is, however, ant to keep this in mind when making aneous determinations of glucose-T_m rue endogenous creatinine clearance. gh glucose concentrations used in these inations will give a slight increase of creatinine levels. The use of yeast the determinations of plasma crea- would solve this difficulty.

plasma "creatinine" value obtained ddition of glucose + acetone is con- le, but not so high as the values found diabetics mentioned above. The reason e that too little acetone was added V) or that the plasma of diabetics ntain other substances giving the Jaffé n, *e. g.* other ketone bodies.

SUMMARY AND CONCLUSION

iabetics with raised blood sugar values tonemia, very high plasma »creatinine" are found. This is due to the high e and acetone levels, as it is shown th glucose and acetone are "pseudo- ines" of considerable importance. ver, also some other substances may role, *e. g.* other ketone bodies.

pointed out that plasma "creatinine" to be read after 10 minutes.

At very high glucose and acetone levels, most of the "pseudocreatinine" may be removed by the aid of Lloyd's reagent. The remainder is of no practical importance.

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PRELIMINARY DATA ON THE COMPOSITION AND CERTAIN PROPERTIES OF HUMAN CHYLOMICRO

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When studying lymph from a patient with a fistula of the thoracic duct, Frazer (1949) observed that the chylomicrons in the lymph differed from those in the blood in that the former migrated electrophoretically with the albumin fraction, the latter with α- and β-globulin fractions. His electrophoresis experiments were performed in a capillary layer. The mobility of the particles was studied with the aid of a dark field microscope. These investigations have not been extended, probably because of the difficulty in obtaining human lymph.

The present paper is concerned with results of an investigation of fat particles isolated from chylous ascitic fluid, obtained from a patient in whom the thoracic duct was more or less completely obstructed by leukemic infiltrations. Laparocentesis had to be performed every few weeks and about 10 litres of milky fluid was aspirated each time. The lipid content of the fluid[1] was about 3 per cent.

METHODS AND RESULTS

Electrophoretic studies were carried out in a Tiselius-Svensson apparatus at pH 7.6 (phosphate-sodium chloride buffer, ionic strength 0.2).

The fat particles were eliminated from the by the method of Brown (1953) (flocculation protamine sulphate), which proved just as u in the study of human ascitic fluid as of lymph.

The electrophoretic patterns of the patie serum and of the ascitic fluid from w the chylomicrons had ben isolated sho relatively good agreement (Fig. 1 a and although the concentration of the β-glob was somewhat lower in the ascitic fluid t in the patient's serum.

Electrophoretic patterns of aliquots had been centrifugated at 5000 r.p.m. v also studied. The milky sample was suc off from the bottom of the centrifuge tub avoid contamination with the thin cre surface layer. This centrifugation was ne sary to eliminate the largest fat part giving rise to convection in the elec phoresis cell on account of their low spe gravity. Comparison of the turbidity on anode side with that on the cathode sho that the fat particles migrate with the a min and α-fraction (Fig. 1 c).

In order to study the charge of the ch microns, equal portions of ascitic fluid serum (collected from a healthy indivi in the fasting state) were mixed and mixture was analysed electrophoretically was clear from the pattern Fig. 1 d) tha charge of the chylomicrons changed in

[1] The samples tested were kindly supplied by Professor, J. Waldenström, M. D., at the General Hospital, Malmö.

vay on mixing ascitic fluid with serum
o as when lymph is mixed with blood
, i. e. after being mixed with serum
ylomicrons from ascitic fluid migrated
ally with the α_2-fraction as is found
m from patients' with postalimentary
ipemia.

lomicrons were also isolated from
fluid by the addition of toluidine blue
otamine sulphate, procedures used
for separating chylomicrons from rat's
(Brown 1953, Borgström & Laurell
Laurell 1953). It should, however,
ntioned that complete flocculation of
particles of this ascitic fluid required
o four times more protamine sulphate
idine blue than did separation of the
icrons from rat's lymph. After mixing
cites with en excess of normal serum,
rticles did not flocculate upon addition
uidine blue just as chylomicrons in
ipemic sera did not flocculate despite
dition of relatively large amounts of
ne blue.

fat particles isolated from ascitic fluid
ans of toluidine blue were obtained in
rm of a milky suspension. This was
ed with the methods used earlier in
udy of chylomicron suspensions from
ymph (Laurell 1953). The results of
nalysis in g per 100 ml suspension
total fat 5.18, total cholesterol 0.27,
holesterol 0.065, phospholipids 0.38,
n 0.09 and carbohydrate 0.0096.

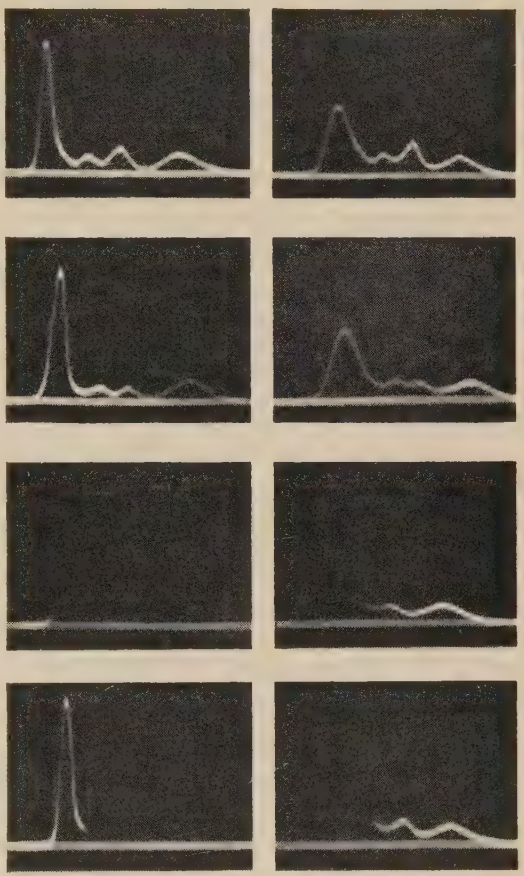

Fig. 1. Electrophoretic pattern of a) serum b) chylous ascitic fluid after elimination of the chylomicrons c) chylous ascitic fluid d) a mixture of serum and chylous ascitiv fluid. Rising boundaries to the left and descending boundaries to the right.

DISCUSSION

it may be assumed that the chylous
fluid examined had accumulated owing
struction of lymph channels draining
estinal tract, it is probable that it consisted mainly of lymph. If this assumption be correct, the observation made in the present study would confirm Frazer's observation that the chylomicrons from lymph differ in electric charge from those of blood. In studies of the electrophoretic mobility of chylomicrons from blood collected from persons with postalimentary hyperlipemia,

Swahn (1953) found that these fat particles regularly migrate with the α_2-fraction.

The observations — that chylomicrons from blood migrate with the α_2-fraction, while chylomicrons from lymph migrate mainly with the albumin fraction — seem to permit the conclusion that the surface charge of the chylomicrons changes on mixing lymph with blood. This assumption was also supported by the fact that in experiments *in vitro* the electric charge of the chylomicrons changed on mixing ascites with serum from healthy individuals.

The difference between lymph and blood chylomicrons with regard to their electric charge and reaction to bases (protamine sulphate and toluidine blue) may be tentatively explained by the assumption that an acid substance is released form the surface of the chylomicrons on mixing the lymph with blood or that a basic substance becomes bound to their surface.

Analysis of suspensions of isolated chylomicrons from human chylous ascitic fluid gave figures not differing essentially from those found in earlier studies of chylomicrons isolated from rat's lymph. Of the total fat, about 5 per cent was represented by cholesterol and about 7.5 per cent by phospholipids. The opinion expressed by Swank & Wilmot (1951) and by Marder, Becker, Maizel & Necheles 1952), that human chylomicrons entirely lack phospholipids could not be confirmed concerning chylomicrons from human chylous ascitic fluid. But their conclusion is drawn on the basis of studies of chylomicrons isolated from blood, further their method for extraction of phospholipids from the chylomicrons questionable. Like rat chylomicrons, chy microns from human chylous ascitic fl contain about 0.2 per cent carbohydrate.

SUMMARY

1. The electrophoretic mobility of chylomicrons from human ascitic fluid mainly the same as that of albumin. addition of serum to chylous ascitic fl the chylomicrons migrate with the α_2-fr tion instead, which is the same mobility t the chylomicrons show in sera with p alimentary hyperlipemia.

2. Chemical analysis of these chy microns showed that they contained *in alia* phospholid (about 7.5 per cent) carbohydrate (about 0.2 per cent).

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EXTRINSIC (P^{32})/INTRINSIC (P^{31}) PHOSPHORUS CLEARANCE RATIO IN MAN

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renal excretion of parenterally in- phosphate has been the subject of sion in recent years since Govaerts) showed that, in dogs, the specific y of inorganic phosphorus is higher urine than in the blood plasma during enous infusion of radio-active sodium hate solution. His results have been d in rats by Friedlander & Wilde), in dogs by Handler & Cohn (1951) children by Ström (1951). Govaerts' retation, according to which the differ- arises from the different excretion nisms for the extrinsic and intrinsic hate, is disputed by Handler & Cohn opinion is that the lag period of the kid- s the main reason for the phenomenon. tly, Östling & Tötterman (1952) have sed the significance of the lag period een able to confirm Govaerts' explana- y infusion experiments in man. As ecific activity of phosphorus is higher urine than in plasma, the clearance adio-active phosphorus/intrinsic phos- s must also exceed 1. Such a condition further support the hypothesis that travenously introduced and intrinsic hate are excreted in different ways.

EXPERIMENTAL

The investigations were effected on surgical patients with normal renal function.

The patients were prepared for clearance determinations in the usual way (Smith 1951). In order to maintain constant radio-activity in the plasma, 0.004—0.006 mc P^{32} was injected intravenously as isotonic sodium phosphate solution, followed immediately by a continuous infusion of the same solution at a rate of 0.00033 mc P^{32}/min. The phosphate solution contained 0.001 mc/ml. Because many different factors affect the elimination of the injected phosphate from the blood, a constant radio-activity of the blood during the time the blood and urine samples were taken was obtained in only 7 of the 11 experiments.

The bladder was catheterized and rinsed with 50 ml of physiological saline solution 15 minutes after the start of the infusion. The urine was then allowed to run from the catheter into collection wessels which, after the rinsing of the bladder with 50 ml of physiological saline solution, were changed every ten minutes. Samples were taken in this way between 15 and 25, 25 and 35, and 35 and 45 minutes after the beginning of the infusion. The blood samples were taken

from a cubital vein at the beginning and end of each urine collection period, at 15, 25, 35 and 45 minutes.

The inorganic phosphorus of the urine and blood plasma were determined according to Fiske & Subbarow, and the radio-activity according to Hevesy and co-workers as described previously (Östling & Gåsström 1952). The clearance ratio P^{32}/P^{31} was computed in the usual way (Smith 1951). The P^{32} values were indicated in counts/min./ml and the P^{31} in mg/ml.

The results of the experiments are compiled in Table I and show beyond doubt that the clearance ratio P^{32}/P^{31} exceeds 1.

Table I.

Case	Sex	Age	Urine collecting time (min.)	Urine flow ml/min.	*Clearance ratio* P^{32}/P^{31}
1	♂	34	25—35	0.8	1.19
			35—45	3.5	1.25
2	♂	79	15—25	3.2	1.24
			25—35	3.6	1.36
			35—45	4.8	1.44
3	♂	59	15—25	14.3	1.22
			25—35	16.6	1.35
			35—45	12.6	1.41
4	♂	39	15—25	13.6	1.13
			25—35	11.5	1.41
			35—45	13.5	1.43
5	♂	56	25—35	1.0	1.14
			35—45	1.9	1.17
6	♂	78	15—25	7.0	1.26
			25—35	5.9	1.35
7	♂	52	25—35	1.1	1.21
			35—45	0.9	1.30

DISCUSSION

The result obtained, that the clearance ratio extrinsic phosphorus/intrinsic phosphorus exceeds 1, confirms the opinion expressed by Govaerts (1948) and by Östling & Tötterman (1952) according to which intravenously injected radio-active sodium phosphate is excreted differently from and more rapidly than the intrinsic phosphate of blood plasma.

It can also be seen from Table I that the methods employed give a greater clearance ratio the later the urine sample is taken, although the radio-activity in the blood remains at the same level throughout. Clearance equilibrium (Michie & Michie 1951), therefore, has not been reached. This is due to the fact that the radio-activity in the plasma does not attain its constant value until 15—25 minutes after the beginning of the infusion, and that we must reckon with a lag period of 20—30 minutes (Östling & Tötterman 1952). The true clearance ratio P^{32}/P^{31}, therefore, is probably higher than the values given in Table I.

As pointed out by Govaerts (1947, 194 and Friedlander & Wilde (1951), the diff ence in the excretion mechanism may be d to the extrinsic and intrinsic phosph having a different physico-chemical state the blood. The binding of intravenously jected radio-active phosphate to a so unknown flotation factor in the blood, sho by Östling (1952), supports this hypothe In addition, it is of clinical interest to n that the renal excretion of radio-active ph phate is strongly reduced in relation to excretion of intrinsic phosphate in patie with diseased kidneys (Ström 1951, Östl & Gåsström 1952, Östling & Tötterm 1952).

SUMMARY

The clearance ratio extrinsic phospho (P^{32})/intrinsic phosphorus (P^{31}) on intravenous infusion of radio-active sodi phosphate solution exceeds 1, which suppo

eory that the introduced phosphate is ed by the kidneys differently from the nic phosphate of the plasma.

present investigation has been aided rant from the Sigrid Jusélius Stiftelse.

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THE RELIABILITY OF THE SCHOLANDER-ROUGHTON METHOD OF MICRODETERMINATION OF THE OXYGE CONTENT OF BLOOD, USING AEROSOL AND SAPONIN AS THE HEMOLYTIC AGENTS

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A report on the occurrence of saponins in the vegetable kingdom and on their hemolysing properties was published by Luft in 1926. Van Slyke & Neill in their method of microdetermination of the oxygen content of blood, employed saponin as the hemolytic agent. Subsequently Van Slyke & Neill (1934), and Hiller, Plazin & Van Slyke (1948) found that the commercial saponin preparations gave considerably lower values in the gasometric oxygen analyses of blood than *e g.* Kahlbaum saponin; hence they were unsuitable for use as hemolytic agents. — In 1944 Vestling and Swerdlow employed Duponol WA and Duponol W-20 as the hemolytic agent in the Van Slyke method. In 1946 Ramsay used oleyl-sodium sulphate and in 1948 Hiller *et al.* urea solution or infusions of senega root, root soapwort and quillaja soap bark. In 1950 Yiengst employed aerosol (dioctyl-sodium-sulfo-succinate) as a hemolytic agent. In 1950 Douglas recommended the use of "Teepol" detergent instead of saponin as the hemolytic agent in the Van Slyke method.

Scholander & Roughton reported in 1942 on a gasometric micromethod of analysing the gases in blood. Their method has certain advantages over Van Slyke's. For this reason and since, as mentioned above, the hemoly effect of the commercial saponins varies, t present investigation is an attempt to stu whether, in the Scholander-Rought method, saponin can be replaced by aero and if so to find the optimal concentrati of the latter, and to check the reliability of t Scholander-Roughton method with aero against the Van Slyke method.

METHODS

A venous blood sample taken with a hypoder needle containing sodium citrate (1 part of 3.8 cent sodium citrate solution ÷ 4 parts of blo was injected under paraffin. The analyses w made simultaneously by both methods within a hours of taking the sample. With the Van Sl method aerosol was employed as the hemoly agent and with the Scholander-Roughton met various hemolytic agents were used. Three para determinations were made with each modificat of the method. The aerosol solution used in Van Slyke method was diluted to 0.25 per c according to Yiengst (1 part of 1.0 per cent aque solution of aerosol ÷ 3 parts of 0.8 per cent po sium ferricyanide solution). Of the hemolytic ag solutions employed in the Scholander-Rough method the saponin solution (saponin BDH, Bri Drug House) was made according to the orig Scholander-Roughton method and the aerosol s tions as follows:

— to three parts of the basic solution (2. potassium ferricyanide, 0.6 g $KHCO_3$ and 10.0

Table I. *Oxygen content per cent (v/v).*

Van Slyke er cent aerosol			Scholander-Roughton											
			II. Saponin BDH				III. 0.06 per cent aerosol				iV. 0.125 per cent aeroso			
	Variation range	Mean	Values	Variation range	Mean	Deviations	Values	Variation range	Mean	Deviations	Values	Variation range	Mean	Deviations
.1	1.3	8.7	14.1	4.0	12.2	+3.5	7.4	2.7	8.5	—0.2	7.9	2.9	9.1	+0.4
.4			12.4				7.9				10.8			
.5			10.1				10.1				8.5			
.8	0.6	11.5	11.1	0	11.1	—0.4	13.1	1.3	12.2	+0.7	11.1	0	11.1	—0.4
.2			11.1				11.8				11.1			
.5			11.1				11.8				11.1			
.0	0.6	3.3	2.8	0	2.8	—0.5	3.9	1.1	3.5	+0.2	3.4	0.5	3.6	+0.3
.6			2.8				3.9				3.4			
.4			2.8				2.8				3.9			
.5	0.3	5.7	5.5	0.6	5.9	+0.2	5.0	0.6	4.8	—0.9	5.5	0.5	5.3	—0.4
.8			6.1				5.0				5.0			
.8			6.1				4.4				5.5			
.8	0.5	10.0	7.1	0	7.1	—2.9	8.1	0	8.1	—1.9	8.8	0	8.8	—1.2
.0			7.1				8.1				8.8			
.3			7.1				8.1				8.8			
.1	2.0	8.7	5.4	0.6	5.6	—'3.6	6.0	0.5	6.3	—2.9	6.5	1.0	6.5	—2.7
.1			5.4				6.5				6.0			
.1			6.0				6.5				7.0			
.3	0.6	5.6	4.5	1.1	5.1	—0.5	5.1	1.7	6.1	+0.5	7.4	1.1	7.4	+1.8
.6			5.6				6.3				6.8			
.9			5.1				6.8				7.9			
.0	0.6	10.2	9.0	0.5	8.8	—1.4	7.4	0.5	7.6	—2.6	6.8	0	6.8	—3.4
.0			9.0				7.4				6.8			
.6			8.5				7.9				6.8			
.1	0.5	7.9	6.8	0.6	7.0	—0.9	7.9	0	7.9	0	8.5	0.5	8.8	+0.9
.1			7.4				7.9				9.0			
.6			6.8				7.9				9.0			
.3	1.2	7.7	5.6	0	5.6	—2.4	5.6	0.5	5.4	—2.6	6.3	0.7	6.1	—1.9
.3			5.6				5.1				5.6			
.5			5.6				5.6				6.3			

dest.) was added one part of 0.25 per cent, art of 0.50 per cent and one part of 1.0 per queous solution of aerosol, giving an aerosol t in the reagent solution of 0.06 per cent, per cent, and 0.25 per cent, respectively. One f the above-mentioned potassium-ferricyanide n mixed with one part of 1.0 per cent aerosol n gave a 0.50 per cent aerosol hemolytic An attempt was made to prepare in the same 1.0 per cent aerosol hemolytic agent from cent aerosol solution. The strong precipitate developed made it necessary to use 1 part of potassium-ferricyanide solution of a concentration half that of the original and 1 part of 2 per cent aerosol solution.

Using the Scholander-Roughton method, 10 analyses were made with saponin, 0.06 per cent and 0.125 per cent aerosol, 9 analyses with 0.25 per cent and 0.50 per cent aerosol, and 8 analyses with 1.0 per cent aerosol. Corresponding control analyses were made by the Van Slyke method.

The various variants of the Scholander-Roughton method and the Van Slyke method were compared statistico-mathematically, using the t test;

Table II. *Oxygen content per cent (v/v).*

	I 'van Slyke' 0.25 per cent aerosol			Scholander-Roughton											
				V. 0.25 per cent aerosol				VI. 0.50 per cent aerosol				VII. 1.0 per cent aeros			
	Values	Variation range	Mean	Values	Variation range	Mean	Deviations	Values	Variation range	Mean	Deviations	Values	Variation range	Mean	Deviations
1.	10.1	0.6	9.7	7.3	1.0	7.8	—1.9	8.3	0.6	8.5	—1.2				
	9.5			7.8				8.3							
	9.5			8.3				8.9							
2.	7.0	0.3	7.1	7.3	2.0	6.5	—0.6	7.3	0.7	7.1	0	7.3	0	7.3	+0
	7.3			7.3				6.6				7.3			
	7.0			5.0				7.3				7.3			
3.	8.5	0	8.5	7.8	1.7	8.9	+0.4	7.3	0.5	7.5	—1.0	7.3	0	7.3	—1
	8.5			9.5				7.8				7.3			
	8.5			9.5				7.3				7.3			
4.	9.1	1.1	9.3	8.6	3.5	9.4	+0.1	10.9	0	10.9	+1.6	12.0	1.1	11.5	+2
	10.0			8.0				10.9				10.9			
	8.9			11.5				10.9				11.5			
5.	11.0	0.9	11.4	8.0	2.9	9.7	—1.7	10.9	1.1	11.3	—0.1	11.5	0.6	11.3	—0
	11.3			10.4				10.9				11.5			
	11.9			10.9				12.0				10.9			
6.	6.4	1.2	6.4	3.5	1.1	4.2	—2.2	4.6	1.2	5.4	—1.0	5.8	1.3	6.4	0
	7.0			4.6				5.8				5.1			
	5.8			4.6				5.8				6.4			
7.	7.3	0.6	7.6	6.9	1.1	7.4	—0.2	9.1	0.6	8.9	+1.3	6.3	1.6	6.5	—1
	7.9			7.4				8.5				5.8			
	7.6			8.0				9.1				7.4			
8.	10.4	0.2	10.5	11.4	0.6	10.9	+0.4	10.3	1.8	10.1	—0.4	10.3	1.2	9.9	—0
	10.6			10.9				10.9				10.3			
	10.6			10.3				9.1				9.1			
9.	11.3	0.3	11.2	7.8	1.4	7.6	—3.6	9.8	0.5	10.0	—1.2	8.5	0.6	8.9	—2
	11.0			6.8				9.8				9.1			
	11.3			8.2				10.3				9.1			

the standard deviation s_{I-VII} was determinated for each method variant from the formula $s = \frac{w}{1.693}$ per cent (v/v). After that the t-deviation was calculated, by pairs, from the following formula:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{s_I^2}{n_1} + \frac{s_{II}^2}{n_2}}}$$

RESULTS

The analysis values [per cent (v/v)], corrected for the effect of the volume of the citrate solution, are given in Tables I and II. In addition, the tables show the vari tion range of the three parallel determin tions of each analysis, the mean of the paral determinations of each analysis, and t deviation of this mean from the correspon ing mean of the Van Slyke parallel dete minations.

Tables III and IV show the dispersion the determination results. It emerged th the dispersion was of the same scale wi both the Van Slyke method and the Sch lander-Roughton method variants, apa

Table III. *Mean range ($\bar{w}$), per cent (v/v).*

n Slyke	Scholander-Roughton		
I	II	III	IV
25 % rosol	BDH saponin	0.06 % aerosol	0.125 % aerosol
0.82	0.74	0.89	0.72

Table IV. *Mean range ($\bar{w}$), per cent (v/v).*

n Slyke'	Scholander-Roughton		
I'	V	VI	VII
25 % erosol	0.25 % aerosol	0.50 % aerosol	1.0 % aerosol
0.57	1.70	0.77	0.80

the variant in which the hemolytic agent 0.25 per cent aerosol; in this case the rsion was somewhat greater.

bles V and VI show the mean deviation e analyses for each modification of the lander-Roughton method from the cor- nding Van Slyke analysis values. The ts obtained with the variant employing per cent aerosol as the hemolytic agent ted least from those obtained with the Slyke method and were of the same as the Van Slyke values.

e results given in Tables V and VI evaluated statistico-mathematically by t-test (Table VII). Within the usual tial error limits the probability with h the Van Slyke and Scholander-Rough- methods lead to the same result was cent for that variant of the Scholander-

Table V. *Average deviation from the corresponding Van Slyke values of the means of the parallel determinations by the Scholander-Roughton method variants.*

saponin -	Aerosol	
	0.06 %	0.125 %
— 0.9 % (v/v)	— 1.0 % (v/v)	— 0.7 % (v/v)

Table VI. *Average deviation from the corresponding Van Slyke values of the means of the parallel determinations by the Scholander-Roughton method variants.*

Aerosol		
0.25 %	0.50 %	1.0 %
— 1.0 % (v/v)	— 0.2 % (v/v)	— 0.4 % (v/v)

Table VII. *t-deviation of the method variants studied, applying the t-test.*

Method variant	Standard deviation (s)	t-deviation
I	0.48	
II	0.44	t_{I-II} 6.89
III	0.53	t_{I-III} 6.79
IV	0.43	t_{I-IV} 4.92
I'	0.34	
V	1.00	$t_{I'-V}$ 10.05
VI	0.46	$t_{I'-VI}$ 2.00
VII	0.47	$t_{I'-VII}$ 3.89

Roughton method in which the hemolytic agent consisted of 0.50 per cent aerosol; with other Scholander-Roughton method variants it was under 0.1 per cent. The difference is statistically significant.

SUMMARY AND CONCLUSIONS

A comparative investigation was made of the determination of the oxygen content of human venous blood by the Van Slyke gasometric macromethod and the Scholander-Roughton gasometric micromethod. The hemolytic agent employed in the former was 0.25 per cent aerosol, and in the latter saponin BDH solution and 0.06 per cent, 0.125 per cent, 0.25 per cent, 0.50 per cent and 1.0 per cent aerosol solutions. The material shown in the tables was treated statistico-mathematically applying the t-test.

It was found from this investigation that the analysis values obtained by the Scholander-Roughton method fitted in with tho obtained by the Van Slyke method, with the usual potential error limits, better wh the hemolytic agent used in the Scholande Roughton method consists of 0.50 per ce aerosol than when the other aerosol conce trations studied and saponin are employe

In addition it was found that the Sch lander-Roughton method with 0.50 per ce aerosol as the hemolytic agent and the V Slyke method with 0.25 per cent as the hem lytic agent correspond to one another.

The authors express their gratitude to D Sakari Mattila, Ph. D., for the statistic mathematical treatment of the material.

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N THE VITAMIN B_{12} CONTENT IN FECES IN CASES OF PERNICIOUS TAPE-WORM ANEMIA

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a recent investigation based on clinical von Bonsdorff & Gordin (1952) shown that the action of dried broad orm alone is definitely anti-anemic in of pernicious tape-worm anemia, like- in Addisonian pernicious anemia if together with gastric juice. Compara- sts with vitamin B_{12} showed that 5— ried tape-worm (50—100 g fresh tape-) is equal to 5 g vitamin B_{12}. These rs conclude that pernicious tape-worm a is probably due to the fact that the tape-worm absorbs considerable nts of B_{12}, hence the host will suffer lack of this important vitamin. In , for instance, fish tape-worm infesta- s extremely common, but pernicious orm anemia is extremely rare and so ddisonian anemia. Experience has n, however, that in Europeans there is rrelation between occurrence of perni- tape-worm anemia and the amount of orm. This is a point against their ption, because — the more numerous arasites, the larger the amount of lost and the greater the predisposition to a. Children infested with tape-worm be highly predisposed to tape-worm anemia because of their small intestinal volume, but that is not the case. In order to find out whether the tape-worm truly absorbs vitamin B_{12} from its host in amounts large enough to cause tape-worm anemia, the vitamin B_{12} content might be determined in serum in anemic patients before and after expulsion of the worm. But a low B_{12} value in serum need not necessarily be due to the tape-worm having absorbed or consumed this vitamin — it may have prevented its resorption in one way or another. Assay of the B_{12} content in feces is a method by which some information may be obtained. In this study, 5 cases of pernicious tape-worm anemia, 2 of intestinal megaloblastic anemia, 2 of addisonian pernicious anemia and some patients with tape-worm but not anemia and some healthy persons were studied for comparison.

METHODS

A sample of feces was dried in an exicator, powdered and extracted with distilled water (1 g feces—100 g water) for 24 hours at + 4° C, and filtered through a Berkefeld filter. The vitamin B_{12} content was assayed in the sterile filtrate with the aid of an E. coli strain, 113—3, kindly supplied by H. Diding of the State Bacteriological Laboratory, Stockholm, Sweden. Dilutions of the filtrate were compared after 24 hours' incubation with dilutions of a known vitamin B_{12} solution. The same

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nutrient broth as that used by Diding (1951) was employed, but no agar was added. As some other substances occurring in feces, at least in high concentrations, might be thought to stimulate the growth of the test organism, series were prepared of another strain, E. coli 5533, which does not grow in the nutrient broth used, not even after addition of vitamin B_{12}. These substances were found to stimulate very slightly the latter strain, *i. e.* only in the first or second test tube. In the first test tube of some of the samples of feces there was sometimes a slight retardation in growth of the test organism, but this was considered of no importance as the border line generally lay in the third to seventh test tube.

RESULTS AND DISCUSSION

The vitamin B_{12} content in samples taken from healthy persons was 0.25 μg on two occasions, and 0.5 μg per 1 g dried feces in three tests. Values between 0.12 and 0.25 were obtained in 4 cases of broad tape-worm carriers without anemia, and 0.5 μg in one case after expulsion of the worm. The result was 0.12 μg in one case of Taeniasis, and 0.06 after expulsion. Before anthelminthic treatment of 5 patients with pernicious tape-worm anemia, the vitamin B_{12} content varied in 9 assays between 0.25 and 2.0 μg, average 0.77 g. The vitamin B_{12} content in the feces of the same patients was 0.015 to 1.0 μg, average 0.4 μg after partial expulsion of parasites. Values between 0.5 and 4.0, average 2.4, were obtained in 4 assays in cases of tape-worm anemia after successful expulsion of the worm. In one case of Addisonian pernicious anemia, the value was 0.1 and in another, in two assays only, 0.06 and 0.015. Broad tape-worm was a secondary finding in the latter patient — the blood picture did not improve after expulsion of the worm and prolonged treatment with large doses of liver was required. The patient's low vi min B_{12} content in the feces may be as ciated with loss of appetite and distinct like of meat and fish. The patient's appet improved in the first week of his stay hospital, and the B_{12} values increased 0.5—1.0 μg.

The results of this study show that vitamin B_{12} content in feces of patients w pernicious tape-worm anemia did not d nitely differ from that in healthy persons tape-worm carriers without anemia. amount of vitamin B_{12} in the feces o patients with intestinal megaloblastic ane (with blind-loops after surgical interferen did not differ from that in normal perso Extremely low values were, however, tained in two cases of Addisonian pernici anemia. This may have been due to the p general condition of these patients, to c plete loss of appetite and the consequen deficient supply of vitamin B_{12}. Fairly la amounts of B_{12} have been found in feces untreated patients with Addisonian pernici anemia, according to the literature (B Castle, Welch, Heinle, Anker, & Epst 1948, and Bethell, Meyers & Neligh 194 The three last mentioned authors give values 1.8—3 μg per 1 g dried feces and other six amounts which suffice to cure disease. Their conclusions are quite plaus — the resorption of vitamin B_{12} is distur in Addisonian pernicious anemia. They not state, however, whether the B_{12} excr is derived from dietary sources or by testinal bacterial synthesis. Similar circ stances are observed in pernicious tape-w anemia with regard to the vitamin B_{12} tent of the feces. — Ludwig has made interesting observation. By means of mi

tical methods, he found large amounts amin B_{12} in the feces of a person who been fasting for a considerable time. ver, there was no antianemic effect of vitamin B_{12} in cases of pernicious a.

twithstanding the fact that a consider- mount of the vitamin B_{12} in the feces een synthesized by bacteria, some of y have been due to food. If the fish tape-worm absorbs large amounts of the vitamin B_{12} contained in the food, it might be expected that the vitamin B_{12} content in the feces of persons infested with tape-worm would be lower. In our investigations it has been observed that the vitamin B_{12} content in the feces varies within the same limits in healthy people, in tape-worm carriers, and in those with pernicious tape-worm anemia.

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BAL-THERAPY IN TRANSFUSION HEMOSIDEROSIS

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In addition to the serum-iron bound to β-globulin, normally about 120 μg/100 ml, the blood serum is capable of binding intravenously administered iron in the same way up to a certain limit (about μg/100 ml), *i. e.* the saturation limit of serum for iron (Holmberg & Laurell, 1945). This latent iron-binding capacity of the serum varies in different diseases (Laurell, 1947). In idiophathic hemochromatosis and transfusion hemosiderosis there is complete or practically complete saturation of the iron-binding protein of the serum (Rath & Finch, 1948). This degree of saturation is so pronounced that it may be used for diagnostic purposes (Rath & Finch, 1948, and Houston & Thompson, 1952). Further, both hemochromatosis and transfusion hemosiderosis are characterised by an accumulation of large quantities of iron pigment (hemosiderin) in the body resulting in progressive changes in several parenchymatous organs which lead, among other things, to liver cirrhosis and pancreatic fibrosis.

An increased deposition of hemosiderin in various organs can be observed also in a number of other pathologic conditions such as pernicious anemia and aplastic anemia. In these diseases, however, it does not result in tissue changes or saturation of the iron-binding protein of the serum. As, in hem chromatosis and transfusion hemosideros the iron-binding protein is saturated it see reasonable to suppose that the amount iron accumulated in the organism exceeded that which can be stored (mos in the reticuloendothelial system) witho causing injurious effects. An observation interest in this connection is that made Holmberg & Laurell (1945), who report that intravenously injected iron begins produce toxic symptoms as soon as saturation limit of the serum has be reached.

Normally the amount of iron excreted the urine is small (0.3—0.5 mg per da despite the fact that 1 g of iron passes kidneys every day in the form of serum ir The daily elimination of iron is practica constant, although studies by Barer & Fow (1949), have shown that the administrat of acidosis (or alkalosis) producing age is capable of increasing (or decreasing) urinary iron.

Theoretically, it cannot be excluded t medication with BAL may increase elimination of iron in the urine, but McCa & Widdowson (1946), were unable demonstrate any such increase in nor subjects treated with this preparation. T

owever, note a twenty-fold increase urinary copper, and a fivefold increase urinary zinc. This enhancement may y be due to these metals being bound erent specific protein fractions. The nding fraction has been described by & Ingelmann (1947), and the copper- fraction by Holmberg & Laurell,

ing by a search of the literature, no on has been directed to the possible of BAL on the iron excretion in s with disturbed iron metabolism. Our on was drawn to this possibility by nt with aplastic anemia in whom trans- hemosiderosis developed after several reatment with blood transfusions. The as been reported elsewhere in detail on, Kullendorff & Ljungberg, 1953). only a caseextract is given.

patient was a 50-year-old farmer who med exposure to toxic agents known nage the blood. In 1945 he was ad- to the medical department of the Central Hospital, because of aplastic . In the course of the years 1945— e received altogether 86 transfusions ut 400 ml each. From June 1949 the narrow function was satisfactory and ther transfusion was necessary. In the of 1950 signs of transfusion hemo- is appeared, with impaired liver func- evere ascites and widespread pigmenta- the skin. Pathologist's report: Hemo- is cutis. There was progressive ration and finally the patient was ally bedridden. In the autumn of 1950 re-admitted to the department where eived 3 courses of BAL at intervals onth. The total dose per course was 1.2—1.4 g. This medication was followed by an increase in the urinary iron. Abdominal paracentesis was also performed on various occasions. From December of that year he gradually improved. It was no longer necessary to perform paracentesis so frequently, and in June 1951 the ascites had disappeared. By the autumn of 1951 he was so much better that he could return to his work as a farmer after 6 years' invalidity. When last examined March 1953 he still felt well, kidney and liver function tests showed no signs of a pathologic condition. There was no ascites, the pigmentation of the skin was much less pronounced and the blood values were normal.

Fig. 1 shows the urinary excretion of iron during the last 2 courses with BAL. Unfortunately, no such determinations were made during the first course. Nor were recordings made of the iron-binding capacity of the serum. The comparatively low value, 163 μg/100 ml for the serum iron, may probably be explained by the fact that, at that time, the patient had ascites and hydrothorax as well as hydremia and, secondly, the serum albumin value was only 4.3 g/100

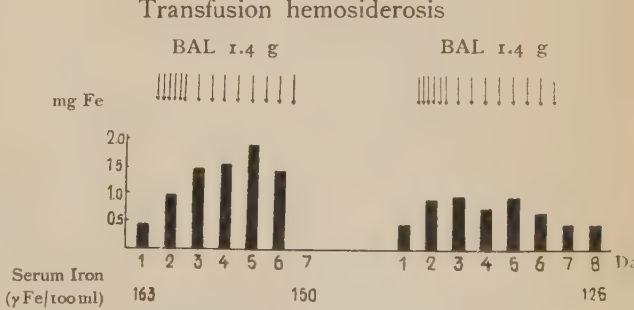
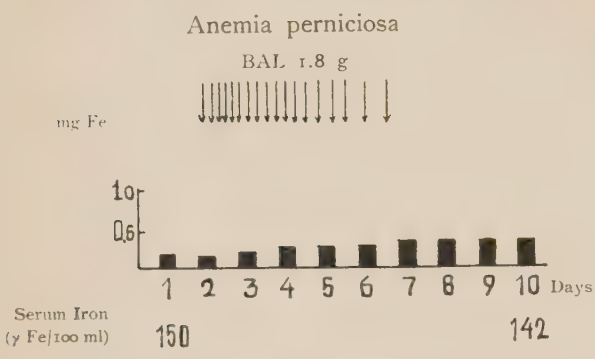


Fig. 1. Daily urinary excretion of iron recorded in a case of transfusion hemosiderosis during treatment with BAL.



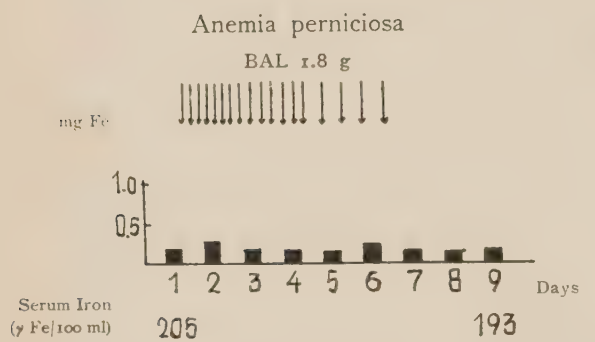
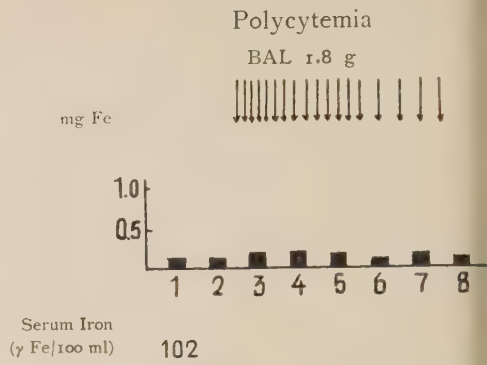


Fig. 2. Daily urinary excretion of iron recorded in two cases of anemia perniciosa during treatment with BAL.



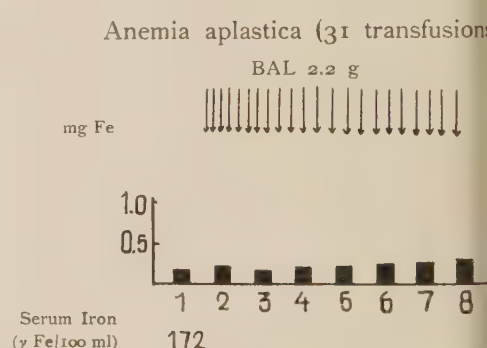


Fig. 3. Daily urinary excretion of iron recorde cases of polycytemia and anemia aplastica du treatment with BAL.

ml. The largest amount of urinary iron per 24-hour specimen was 1.8 mg, *i. e.* five times the normal value.

Similar determinations of the urinary iron during medication with BAL were made in some patients with pernicious anemia and aplastic anemia, where there is disturbance of the iron metabolism and an accumulation of hemosiderin, but no organic damage or increased saturation of the iron-binding protein of the serum. The patient with aplastic anemia had received 31 blood transfusions and died soon afterwards. Postmortem examination showed extensive deposition of hemosiderin but no signs of organic damage such as occurs in transfusion hemosiderosis.

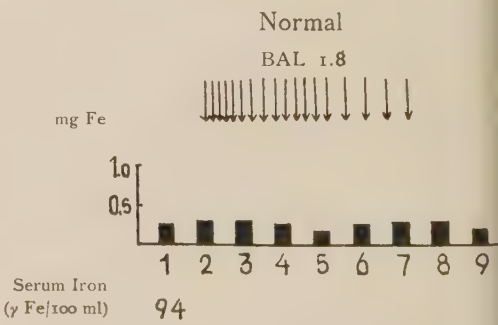


Fig. 4. Daily urinary excretion of iron duri treatment with BAL in healthy male volunteer.

It is clear from Figs. 2 and 3 that in t cases BAL produced no increase in urinary iron. Nor did administration of

ealthy subject (Fig. 4) produce any e in the urinary iron, an observation arlier also by McCance & Widdow- 946).

COMMENTS

ientioned earlier, hemosiderosis (and romatosis) differs from other condi- ith increased deposition of hemo- in the organism by the high degree ration of the iron-binding protein serum and the presence of cirrhosis liver and pancreatic fibrosis. It has n suggested that the complete satura- the serum with iron may be taken n that the amount of iron accumulated e conditions has exeded the storage of the organism. The surplus is then ed in various organs in such quantities exerts toxic effects. Unlike the nor- tored iron, the excess iron deposited rgans can be controlled by medication with BAL. The increased urinary iron observed in the case under consideration during medication with BAL seems to be compatible with this line of thought, especially as the patient made a good recovery after treatment. Judging by a search of the literature available, this is the only case out of 30 on record in which the disease ran such a favourable course.

SUMMARY

Medication with BAL produced an increase in the urinary iron of a man with transfusion hemosiderosis who afterwards gradually improved and after 6 years of invalidity was able to return to his work as a farmer.

The administration of the preparation produced no such increase in patients with pernicious anemia, aplastic anemia or in a healthy subject.

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A COMPARISON OF THREE METHODS, UTILIZING DIFFERENT PRINCIPLES, FOR THE DETERMINATION OF URIC ACID IN BIOLOGICAL FLUIDS[1]

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The determination of uric acid in biological fluids is performed by widely different methods in different laboratories and these methods have not given the same results for normal subjects. We therefore felt that it would be reasonable to compare three of the methods which are fundamentally different.

METHODS

The colorimetric method for quantitative determination of uric acid in blood was introduced by Folin (1912) and numerous modifications have been suggested in the course of time. To day it is the method most commonly used in Anglo-Saxon countries. The principle is as follows: Proteins are removed and phosphotungstic acid is added under fixed conditions, giving a deep blue color with uric acid. The intensity of the color produced indicates the quantity of uric acid in the specimen. We employed the modification advocated by King (1951), where 0.2 ml of serum is required for a single determination. The colorimetric determination was performed in a Beckman spectrophotometer.

The Brøchner-Mortensen (1937) titrimetric method, where the uric acid is measured by its ability to reduce potassium ferricyanide, is the chief method employed in Scandinavian countries. Proteins are removed, potassium ferricyanide is added in excess and the nonreduced part determined by iodometric titration. For a sin determination 5 ml of serum are required.

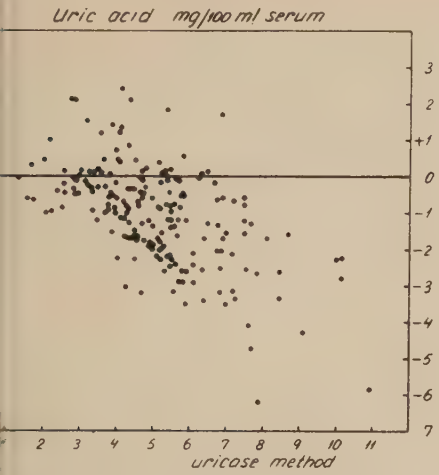
The uricase method was published by Prætor (1949). Its principle, as first described by Kalck (1947), is as follows: the absorption by uric a is maximal at 292.5 mμ in the ultraviolet ran It is determined in a sample of diluted serum fore and after addition of a small quantity of enzyme uricase which splits uric acid. The decl in light absorption at the wavelength mentio is an indication of the quantity of uric acid in sample. For a single determination 0.1 ml of ser is required. The uricase was supplied by E. P torius, M. D., Department of Pharmacology, U versity of Copenhagen.

Three laboratory technicians worked on tests, each performing it by a different method the same sample. They were unaware of results which might have been obtained by different methods. Duplicate determinations w performed in all cases with the uricase meth whereas in most cases only single determinati were made with the other two methods.

The accuracy of a single determination established by analysing 20 aliquots from the s pooled serum. The standard deviation was he found to be about 3 per cent for the colorime to be about 3 per cent for the titrimetric an be about 2 per cent by the uricase method.

The uricase method is specific for u acid whereas both the colorimetric and ti metric methods have been criticized for th lack of specificity. The only objection the uricase method is that it requires a ra

[1] This work was supported by a grant from The National Organization Against Rheumatic Diseases, Copenhagen.



Uric acid determinations in 188 different samples, employing the enzymatic and colorimetric methods.

ve instrument and a highly purified as yet only produced in few places, e other two methods may be carried means of the apparatus available in pital laboratory.

e last mentioned facts serve to stress ue of finding a relation between the obtained by the three methods.

RESULTS

total of approximately 200 samples n we determined the uric acid by the ree methods. The blood was obtained rom normal subjects and partly from in the Medical Department of the Hospital, Copenhagen.

l shows the results of 188 simultane- erminations by the uricase method torius and the colorimetric method . Each pair of results in milligrams is indicated by one dot, the abscissa the value found by the uricase and the ordinate the difference between the value found by the colorimetric method and by the uricase method. The dots representing all tests in which the two methods gave the same result will then be situated in the zero-axis. All tests in which the colorimetric method gave a higher value are represented by a dot above the zero-axis, and all tests in which the colorimetric method gave a lower value than the uricase method are illustrated by a dot below the zero-axis. This diagram shows in the first place, that the dots are scattered vertically, indicating poor conformity between the two results. In the second place, the great majority of the dots are situated below the zero-axis, meaning that in most instances the colorimetric determination gave a lower result. This tendency to low results is most marked in the case of the high values.

Similarly Fig. 2 illustrates the results from the 233 specimens determined simultaneously by the uricase method and by the titrimetric method. The first thing which is apparent in the diagram is that the dots are scattered,

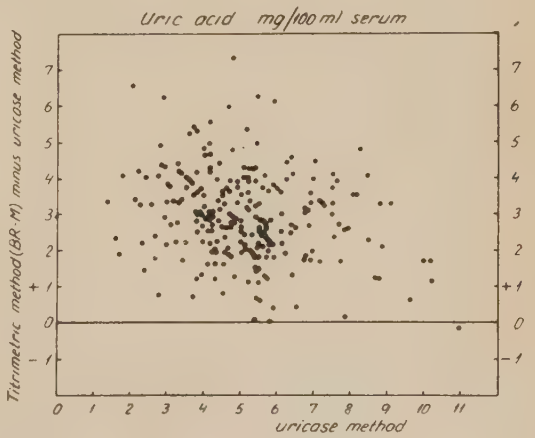


Fig. 2. Uric acid determinations in 233 different serum samples, employing the enzymatic and titrimetric methods.

and also that practically all the dots are above the zero-axis. In other words, the titrimetric method gives a value higher than that obtained by the uricase method.

Finally it must be mentioned that in this series, in which all three methods were used for determining the same specimens of serum, an equally poor conformity was shown in both normal subjects and in patients.

DISCUSSION

We did not succeed in demonstrating any correlation between the results of determinations made by the specific enzymatic method and by the two less specific colorimetric and titrimetric methods, as the two diagrams show that in both instances the scattering is so marked that it is impossible to calculate one value from the other, either by adding or subtracting a fixed value or by multiplying by any factor.

Therefore it is recommended that the less specific methods should be abandoned and that the specific uricase method should be employed.

Further, our results indicate that investigations performed by the older methods on the normal level for serum uric acid, on the distribution in body fluids, on the urinary clearance etc. have a very limited value.

SUMMARY

On approximately 200 samples of ser from patients and normal subjects uric a was determined by three different metho

1) an enzymatic, differential-spectroph metric method (Prætorius).
2) a colorimetric method (Folin) and
3) a titrimetric method (Brøchner-Mort sen).

No correlation was found between t results of the specific enzymatic method a the results of the less specific methods. T use of the enzymatic method is reco mended.

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A CLINICAL METHOD FOR DETERMINATION OF PLASMA GALACTOSE IN TOLERANCE TESTS

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(Received for publication October 24, 1953)

mination of galactose in plasma is clinical laboratories, especially as a tion of the liver function test (Coltek & Kendall, 1946, Gottlieb 1934, 1937, Stenstam 1946) introduced by 1906), by which the influence of the on the result can be eliminated. r, this procedure has not become so used at it deserves, chiefly because e determinations of galactose in the f concentration to be examined have practical difficulties in most labora-

letermine galactose in plasma, it is ry to use the reducing property, as ct and specific method is available sugar. When a proper plasma protein ating agent is used, glucose is the educing substance, the others being in low and fairly constant concen- . Therefore the increase in non-fer- e reducing substances found after tration of galactose has been con- a measurement of galactose in plasma eltoft & Kjerulf-Jensen, 1943, Lar- Sveinsson 1940, Raymond & Blanco The reliability of these methods is ied by the fact that live yeast used fermentation augments the rest reduction in an uncontrollable way (Ege & Rasmussen, 1919).

As a pure and specific glucose oxidase (Notatin) has been obtained from *Penicillium notatum*, and as this does not suffer from this defect, it has become possible to improve the galactose analysis.

We considered a colorimetric determination of the reduction most suitable, as it yields great accuracy at small concentrations, but a titration method like Hagedorn-Jensen's (1935) affords sufficiently accurate results at concentrations beyond 15—20 mg per cent.

REAGENTS

a. Notatin (Boot's). *b.* KH_2PO_4, m/5 solution. *c.* $Fe_2(SO_4)_3$, analyzed (Baker), 17 per cent solution (a filtered stock solution which will keep well when not heated is diluted 1:7 before use). d. $BaCO_3$, finely pulverized. *e.* Na_2SO_4, in solution saturated at room temperature. *f.* Reduction reagent: I. $CuSO_4$, 5 H_2O, 4 g; Na_2SO_4, anh., 36 g, made up with water to 200 ml. II. Na_2CO_3, anh., 24 g, Na_2SO_4, anh., 144 g, $NaHCO_3$, anh., 16 g, KNa-tartrate, 12 g, made up with water to 800 ml. I. and II. are mixed before use in the ratio 1:4. *g.* Chromogenic reagent: Ammonium molybdate, 50 g, sulfuric acid, concentrated, 21 ml, sodium arsenate, 3 g, made up with water to 475 ml. Galactose puriss. (Sandoz).

All reagents are of analytical grade, redistilled water is used.

PROCEDURE

2 ml of heparin plasma and 2 ml of phosphate solution (b) containing ½ mg of Notatin (a) are pipetted into a 100 ml Kjeldahl flask which, when stoppered, is placed in a water-bath in which the samples can be continuously shaken, at 38° C for 1½ hours.

The proteins are precipitated with 16 ml of ferric sulfate solution (c) and 3 g of barium carbonate (d), and the mixture is vigorously shaken. If, in spite of shaking, the liquid remains acid as controlled with litmus paper, precipitation is incomplete and a spatula of barium carbonate is added and the flask is shaken again. The solution is filtered through a reduction-free filter (S.S. 597) into a centrifuge tube.

0.2 ml of saturated sodium sulfate (e) solution is added to the filtrate, this is mixed and, after a few minutes, centrifuged for 5 minutes at 3000 r.p.m.

2 ml of the filtrate is pipetted into test-tubes with screw-on stoppers (see below) and 2 ml of the reduction reagent (f) is added. The test-tubes are closed with the screw-on stoppers and placed in a briskly boiling water-bath for 30 minutes. When they have been cooled down, 2 ml of chromogenic reagent (g) is added and each test-tube is vigorously shaken till effervescence ceases. The contents are poured into a preparation vessel (30 × 100 mm) and the test-tube is rinsed with 20 ml of water, this is also poured into the preparation vessel. After 1½ hours the solution is ready for colorimetry.

DISCUSSION OF THE METHOD

Fermentation

A glucose oxidase was first isolated from a penicillium strain by Müller (1926). Keilin & Hartree (1948) have investigated the properties of the glucose oxidase Notatin obtained as a pure enzyme from *Penicillium notatum*.

The pH-optimum of the enzyme is about 5.6. This reaction is obtained approximately by adding to the plasma sample an equal quantity of 0.2 molar primary potassium

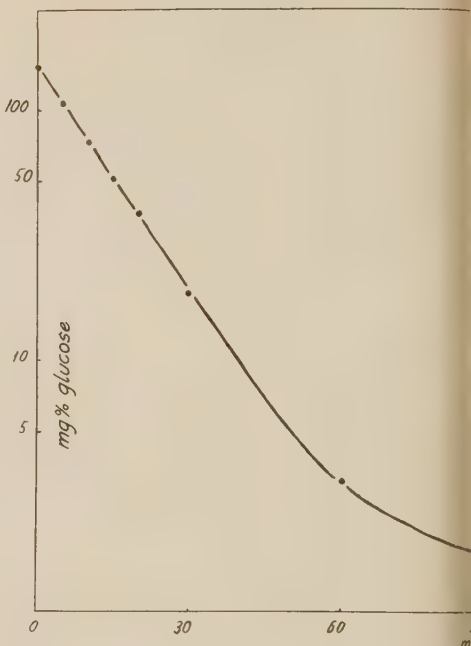

Fig. 1. Decomposition of glucose with Notati

phosphate. The temperature optimum 38—40° C, and consequently the fermen tion is best performed in a thermostat. T sample has to be shaken to supply the re tion with oxygen.

Under these conditions the oxidation glucose to gluconic acid follows the cu shown in Fig. 1. It is seen that the proc at first follows a logarithmic curve and comes slower at low concentrations.

It was considered most convenient to the enzyme work for a period 10 times half-period of glucose oxidation in system. The amount of glucose is t brought down to values of the same or of magnitude as the error of the meth regardless of the initial concentrat (Table I).

I. *Carbohydrate solutions treated h Notatin for various periods.*

inutes) ..	0	10	60	90	120
in plasma %)	148	79	3	2	—
in plasma %)	103	39	—	3	2
in water %)	110	52	—	2	2
in plasma %)	102	—	—	104	103

half-period of glucose oxidation with of Notatin (batch N 14)[1] was 10 , and therefore 1½ hours oxidation d for the analysis.

rate of oxidation of galactose by is by Keilin *et al.* stated to be 1.5 le of that of glucose, accordingly we nable to see any disappearence of se during the period of fermentation I). The presence of galactose does luence the splitting of glucose by

Precipitation of proteins

er's method (1932) was chosen for reasons. It is not influenced by the resent, it does not produce any redu- bstances, and we have found it to w rest reductions in plasma. The ation of barium ions is necessary, as e sulphate ions present in the reduc- agent.

t kindly placed at our disposal by Boot's rugs, Ltd., Nottingham, England, who at the preparation was 11 years old; the eparation is said to be 10 times more

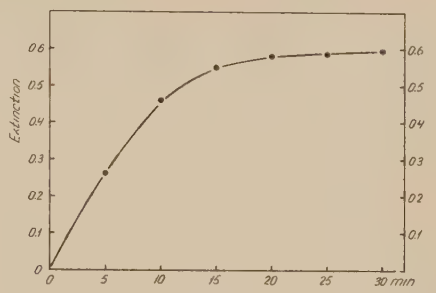

Fig. 2. Influence of boiling time on the reduction process.

Reduction

Somogyi's modified copper reagent (1952) was used, since it is less autoreducing than Somogyi's original one because of its lower content of copper and tartrate. The reduction is carried out in test-tubes (14 × 140 mm) provided with screw-on stoppers (Eltang tubes)[1], preventing evaporation during boiling; this has been ascertained by weighing tubes with contents before and after boiling.

The time required for boiling in glucose determinations with similar reduction reagents is stated to be 10 minutes, but as galactose reduces more slowly, especially with this rather weakly alkaline reagent, a longer boiling time is required. It has been shown that the quantity of cuprous oxide formed increases rapidly during the first 20 minutes. A more protracted boiling only effects slight increase, and small changes in boiling time are therefore insignificant (Fig. 2).

[1] Rinsed in sulpha detergent (even in high concentrations without reducing property), then boiled for 30 minutes in distilled water.

Care should be taken that the liquid in the water bath is above that in the tubes, and the filtrate and the reagent should be mixed carefully before boiling.

Photometry.

The cuprous oxide formed by the reduction can be determined colorimetrically by Nelson's arsenic reagent (1944).

When the latter has been added, the mixture should be vigorously shaken, partly to expel carbon dioxide, partly, and especially, to have all the cuprous oxide that has been deposited on the tube completely removed. It has been previously stressed that the color develops almost instantaneously and remains unchanged for a prolonged period (Somogyi 1952). With the method employed here the intensity of the color increases by about 15 per cent during the first hour (Fig. 3), after which it remains constant for at least 48 hours when the sample is kept cold and in darkness.

Before the results were read, all analyses were diluted with 20 ml of water; the extinctions thus become suitable for the photometer (Beckmann, Model DU) when t reading is taken at the wave-length 585 m The absorption maximum is about 750 m (Fig. 4), but readings here would requi much higher dilutions. Control readings maximum showed the same error as tho taken at 585 mμ.

Fig. 3. Intensity of color developed as function of time.

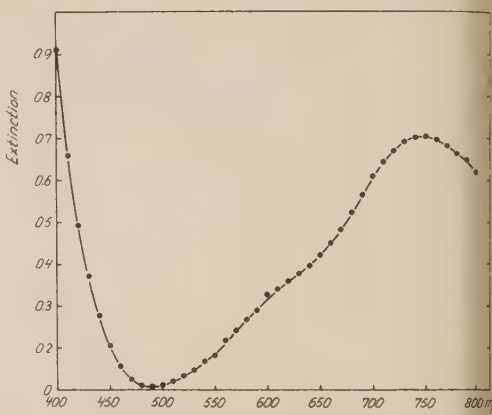

Fig. 4. Spectrum of the color.

The standard curve showing the extincti coefficient as a function of galactose conce tration with the dilutions used was prepar with aqueous galactose solutions and co trolled with filtrates of fermented plas with known contents of galactose. T extinction coefficient is directly proportio to the concentration up to about 500 per cent. The conversion factor from extin tion coefficient to galactose in mg per c in plasma was found to be 150 with standard deviation 5 .

The unfermentable rest reduction

In galactose tolerance tests the galact concentration is measured in plasma agai fermented plasma taken before the admi

n of galactose. In this manner a cor- is made for the rest reduction in- the small fraction of non-fermented . Occasionally there is found no able rest reduction higher than what e accounted for by non-fermented (2—3 mg per cent), whereas plasma st subjects will show a somewhat rest reduction (averaging 5 mg per t the outmost 10 mg per cent).

obvious that the method is only valid est reduction is essentially unchanged hout the test, and changes that actually annot be observed. However, Harding nt 1932/33) have observed no signi- changes in rest reduction in such ctose test, when they removed the se with a strain of galactose fer- g yeast. In our procedure the rest on is so small that even relatively hanges will not influence the results antly.

get an impression of the stability of duction found by our procedure, 10 samples were drawn during the first fter injection of 100 ml isotonic saline n (under the experimental conditions lly used in intravenous galactose ce tests). In this experiment the duction was found to be 5 mg per ith a standard deviation of 1.4 mg nt. Ten duplicate analyses of a single sample from another subject showed reduction of 3 mg per cent with a rd deviation of 1 mg per cent. There significant difference between the rd deviations in these two experi-

39 duplicate analyses in various s the rest reduction averaged 5 mg per cent with a standard deviation of 3 mg per cent. This variation is significantly greater than the standard deviation of the analysis proper and must be due to individual differences in the rest reduction.

Higher and more varying rest reductions were found when oxalate was used instead of heparin.

Reliability of the method

In plasma with a known content of galactose, the standard deviation at concentrations of about 100 mg per cent was found in repeated experiments with ten duplicate analyses to vary between 1 and 2 mg per cent; at concentrations of about 10 mg per cent the standard deviation varied from 1 to 1.5 mg per cent.

The error involved in the use of pipettes and in the photometer readings amounts to about 1 per cent in all; this explains the somewhat higher standard deviation at higher concentrations.

If the considerable dilution before the colorimeter readings is omitted and the absorption maximum is read, small quantities of plasma, *e. g.* capillary plasma may suffice, but we have found that the standard deviation of that procedure is considerably greater. It seems most likely that it is the reduction procedure that takes place with less accuracy when small amounts of substance are involved, presumably because reducing substances are liberated from the ordinary glassware in use in the Eltang tubes. The standard deviation in such experiments could not be obtained lower than corresponding to 30 microgrammes.

SUMMARY

A method of determining galactose in plasma is described; it depends on fermentation of glucose with a specific glucose oxidase, Notatin, precipitation of the proteins with a reagent that yields nearly "true sugar", and colorimetric determination of the non-fermentable reducing substance.

The standard deviation of the method was found to be about 2 mg per cent at concentrations of about 100 mg per cent and about 1 mg per cent at concentrations of about 10 mg per cent.

AKNOWLEDGMENT

We are indebted to Dr. E. Bojesen for help in elaboratting the fermentation method, and for his kind and willing advice.

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A SIMPLE METHOD FOR THE PREPARATION PROTOPORPHYRIN FROM WASHED ERYTHROCYTES

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stein (1947) described a method for reparation of protoporphyrin from cytes. The method is based on the ion of hemin from acetone-washed obin powder with 10 per cent oxalic methanol, and removal of the iron nd esterification of the porphyrin by is bubbling of hydrochloric gas h the solution after addition of crystal- rrous sulphate.

sen (1953 a) has found that the heme- linkage is easily broken by methanol t addition of acid, and that the heme can be extracted almost completely hemoglobin powder by continuous ion with methanol in a Soxlet appa- Then the methyl ester of protopor- is easily prepared with hydrochloric d ferrous sulphate.

METHOD

ted blood from horse, swine, rabbit or man (other species have not so far been in- ed).

erythrocytes are centrifuged down and a couple of times with 1 per cent NaCl, 1 10 times the volume of acetone is added. ecipitates the proteins and extracts the main of the acetone-soluble lipids. The hemo- owder is suspended in about 5 volumes of l (Merck p.a.) an shaken for a couple of centrifuged and the Hgb-powder is brought into a filter cup and extracted with new methanol in a Soxlet apparatus for about 4 hours or until the extract is almost colorless. Crystalline ferrous sulphate is added, and HCl-gas is bubbled through for a couple of minutes. The temperature rises to around 50° C and the color of the solution changes from deep red to red-violet. A small amount of chloroform is added and the solution is brought into a separatory funnel. Distilled water is added to separate the phases. The water phase is washed with small amount of chloroform until all of the porphyrin has been brought into chloroform. The chloroform solution is washed once with distilled water, once with 10 N NH_3 and twice with distilled water, filtered through a chloroform-moistened filter paper and concentrated to a small volume on a water bath, excess of hot methanol is added and the last traces of chloroform evacuated. The solution is left at room temperature for crystallization.

The methyl ester crystallizes readily and with high yield. The mother liquor is only weekly colored but still another small crop can be obtained by keeping in a refrigerator over night. The crystals are centrifuged down and washed a couple of times with cold methanol, and recrystallized from petrole ether/chloroform, washed with petrol ether and dried in a vacuum dessicator.

The identity of the porphyrin has been established by the following observations: The mehyl ester crystallized in small rosettes of short needles. The melting point of the ester was 222° C (uncorrected). On chromatography of the ester on MgO grade III according to Nicholas (1951) only one frac-

tion occurred, which was eluted with chloroform/methanol 100/3. Paper chromatography according to Chu, Green & Chu (1951), gave one spot with an Rf-value of 0.84 corresponding to protoporphyrin dimethylester. Hydrolysis of the ester with 7 N HCl and chromatography on paper using 2—6 lutidine (Hopkins & Williams Ltd., London) according to Eriksen (1953 b) gave only one spot with an Rf-value of 0.84 corresponding to a dicarboxylated porphyrin.

RESULTS

The preparation of the Hgb-powder is of great importance for the extraction of the heme. It is necessary to wash the erythrocytes at least twice with isotonic or slightly hypertonic sodium chloride solution to remove all of the plasma present, to get a smooth and fine protein precipitate with acetone, which makes it possible for the methanol to contact all of the hemoglobin and split the heme-globin linkage.

In the method described no precipitate forms during the washing procedure, no oxalic acid and oxalic acid methyl ester is present, and the washing procedure is simple and time-sparing. It has been found unnece sary to chromatograph the porphyrin es on calcium carbonate or any other adsorbe The porphyrin ester crystallizes well in hi yields.

SUMMARY

A method for the preparation of pro porphyrin dimethyl ester from washed e throcytes with high yield has been describe

The method is based on the splitting the heme-globin linkage with metha (Eriksen, 1953 a), and removal of the ir and esterification of the porphyrin as d scribed by Grinstein (1947).

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TEMPORARY OCCLUSION OF INTERATRIAL SEPTAL DEFECT IN MAN

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Aided by a grant from the Swedish Medical Research Council

(Received for publication October 27, 1953)

ical techniques for occlusion of inter-eptal defects (ASD) have been de- (Bailey, Downing, Geckeler, Likoff, rg, Scot, Janton & Redendo-Ramirez Cross, Pomeranz, Watkins & Gold- 1952, Hufnagel & Gillespie, 1951; , 1953; Murray, 1948), but the in- ns for surgical correction of the defect l not well defined. The role of the ed pulmonary flow in cases with ASD pulmonary hypertensions that some- levelop is obscure (Wood, 1952). In o study this influence and obtain more ation on the acute effect on the cir- n of closure of the septal defect, a technique has been designed. This describes the technique that has been in one case.

ecial catheter with three channels, one at the tip and two at the sides 1 and rom the tip has been designed.[1] (Fig. Two special rubber balloons are placed catheter in such a way that each can be inflated separately through o channels ending proximately. Each can be inflated with 25 ml fluid.[2]

nufactured by the United States Catheter y, Celen Falls, N. Y.

H. E. Hanson cooperated in the devel- of this instrument.

Fig. 1 b shows the catheter with the balloons inflated.

The catheter has an outer diameter corresponding to a number 10 regular heart catheter. It can be introduced through any vein

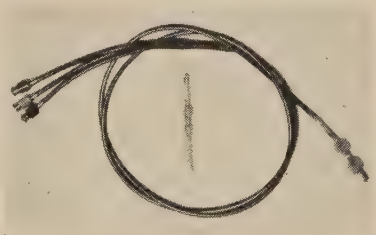

Fig. 1 a.

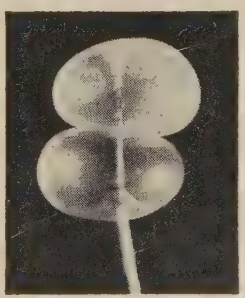

Fig. 1 b.

Fig. 1 a. The Varnauskas 3 channel catheter. b End of same with the two balloons inflated each containing about 15 ml fluid. Note that the balloons are close together. The third channel opens at the tip of the catheter thus allowing blood sampling and pressure recording distally to the balloons.

large enough to permit use of this size catheter. It may be of advantage to introduce the catheter through the saphenous vein as it is easier to pass an interatrial septal defect that way. After the tip of the catheter has entired the right auricle it is pushed further through the septal defect, the left auricle and preferably out in a pulmonary vein. The distal balloon is then inflated under fluoroscopic control with 30 per cent diodrast with about 15 ml and the catheter withdrawn with the balloon inflated. When the balloon rests against the edge of the septal defect it is impossible to withdraw the catheter further. The proximal balloon is then inflated in the same way. It will then obturate the defect on the right auricular side. The two balloons can be seen moving with the heart beat but it is otherwise impossible to displace the catheter or the balloons if the technique has been properly employed. When hemodynamic equilibrium has been reached it is possible to study the effect of exercise or of drugs with the septal defect closed. The designed catheter allows the registration of the left auricular pressure with the balloon inflated and the defect closed. For determination of cardiac output another catheter must be placed in the pulmonary artery which is easily done.

After the completion of the studies the diodrast in the proximal balloon is withdrawn and that balloon totally emptied. The distal balloon is then slowly deflated under fluoroscopic control and constant slight pulling of the catheter. When the size of the balloon corresponds to the size of the septal defect it is possible to withdraw the balloon to the right atrium. The amount of diodrast

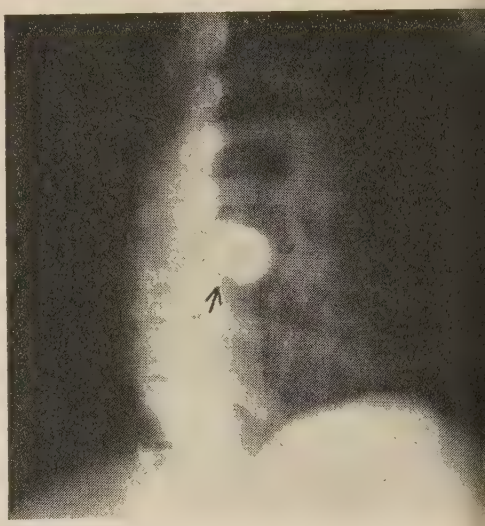

Fig. 2. The Varnauskas catheter passed thro an interatrial septal defect with the balloons flated, one in each side of the atrial septum. distal balloon was withdrawn through the de containing 7 ml diodrast, corresponding to a meter of the balloon of about 2 cm.

still in the balloon is determined. The size the septal defect can then be determi from the size of the balloon inflated with same amount of fluid after the study. Fi shows the balloons inflated in a case of A

The size of the balloons during maxi inflation is about 15 ml each which does interfere with the blood-flow through atria. It will be possible to close only fa small and well defined septal defects, the described technique. The investiga will however give a definite answer if defect is larger, which is of paramount terest for the surgeon.

The technique has been used without difficulty and without any subjective s ptoms from the patients. The procedure

complications as the closure of one of nonary arteries described by Carlens, & Nordenström (1951).

results of hemodynamic studies with hnique will be published separately.

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EXCRETION OF NORADRENALINE IN URINE IN HYPERTENSION

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Biologically active catechol amines occur normally in urine as shown by Holtz, Credner, & Kroneberg (1947). The statement of these authors that the "Urosympathin" probably contained noradrenaline, adrenaline and hydroxytyramine has been confirmed (Euler & Hellner, 1951).

In a group of 20 healthy medical students Euler & Hellner estimated the output of noradrenaline at approximately 30 μg per 24 hours with a range of 9.6—63 μg.

During certain physiological conditions the excretion of noradrenaline in urine may be considerably increased. In heavy muscular exercise for example (Euler & Hellner, 1952), the output may reach 0.40 μg per minute as compared with 0.02—0.06 μg per minute during rest or sedentary activity.

Alterations in the catechol amine output of patients have also been noted following the administration of ACTH protein and peptides and cortisone (Luft & Euler, 1952). There was a striking decrease in excretion of noradrenaline but only slight changes in the adrenaline output.

In cases of phaeochromocytoma the urinary output of catechol amines is usually strongly increased, up to 3000 μg per 24 hours (Engel & Euler, 1949, Euler, 1951, Goldenberg, 1951, Euler, Lund, Olsson & Sandblom, 1953, Burn, 1953). The proportion of n adrenaline and adrenaline in the urine well correlated with the relative content these substances in the subsequently remo tumour. After removal of the tumour output of catechol amines in urine decrea from as much as 2 μg per minute down normal. For comparison it might be m tioned that during infusion of about 15 noradrenaline per minute, which cause rise of blood pressure of some 30—40 Hg, the additional output per minute noradrenaline was about 0.30 μg per mi (Euler & Luft, 1951).

It would therefore seem of importa to study the catechol amine output in u in a large series of patients with esse hypertension.

Goldenberg, Pines, Baldwin, Greene Roh (1948) have reported that hyperten patients responded to a given dose of adrenaline with a higher rise in blood p sure than normotensives.

MATERIAL AND METHODS

The material used consisted of the lected 24 hour urine from 500 hyperten patients from various hospitals in Swe In many cases phaeochromocytoma

d from the clinical picture but no h verified tumour has been included resent material. All cases had hyper- of unknown origin, selected at but corresponding to the "essential" No detailed classification was ed, however.

ystolic blood pressure was in no case an 150 mm Hg and was often about n Hg. In those cases where more e sample was sent for analysis only hest excretion figure has been in- n the material.

urine was acidified to pH 3 during ection, in order to minimize losses idation, and sent to the laboratory ysis. A delay of 48 hours before the on procedure could be started fre- occurred but such a delay is not nied by any appreciable loss of as control experiments with fresh ept at pH 3 and room temperature 8 hours have shown.

method of preparation was that de- previously by Euler & Hellner An aliquot of 100—300 ml accord- he volume available of the collected urine was used for the extraction. adsorption on aluminum hydroxide ples were treated by acid hydrolysis nin. at 100° and pH 1.5. This treat- splits certain conjugated catechol (Richter, 1940, Beyer & Shapiro, vithout causing appreciable losses in ogical activity by racemisation (Kisby u, 1951). The yield in the extraction re is estimated at approximately 75 t, which is the amount recovered ded noradrenaline. No corrections has ade for the losses during extraction.

The extract was assayed on the cat's blood pressure against a l-noradrenaline hydrochloride standard. Since the urine catechol amines chiefly consist of noradrenaline and the activity of adrenaline is usually ½—¼ of that of noradrenaline on the cat's blood pressure, the noradrenaline figure obtained in this way generally deviates less than 10 per cent from the true figure obtained by separate estimation of adrenaline and noradrenaline, using the cat's blood pressure in conjunction with the fowl's rectal caecum.

The following typical example illustrates this point:

Noradrenaline equivalents on cat's blood pressure = 4 (a) μg per cent.
Noradrenaline equivalents on hen's rectal caecum = 20 (A) μg per cent.
Adrenaline/noradrenaline activity ratio on cat's blood pressure = 0.3 (q).
Adrenaline/noradrenaline activity ratio on hen's rectal caecum = 30 (Q).

Using the formula for calculation of noradrenaline and adrenaline (Euler 1949):

$$\text{Adr.} = x = \frac{A - a;}{Q - q} \quad \text{Noradr.} = y = A - xQ$$

one obtains:

Noradrenaline	= 3.84 μg	per cent
Adrenaline	= 0.54 μg	» »
Total catechols	= 4.38 μg	» »

The noradrenaline equivalent figure obtained from the cat's blood pressure (4.0) thus differs only insignificantly from the correct figure (3.84) and it was considered that the approximation involved was of no great importance for the general result. The error of assay on the cat's blood pressure

Table I. *Estimated noradrenaline excretion in urine in 500 cases of hypertension.*

Noradrenaline excretion µg per 24 hours	Number Females	Number Males	Total number	Per cent of total material		
				Females	Males	Total
<28.8	85	42	127	24.9	26.4	25.4 %
28.8—57.6	134	70	204	39.5	44	40.8 »
57.6—86.4	67	20	87	19.6	12.6	17.4 »
86.4—115	26	14	40	7.6	8.0	8.0 »
115—144	9	9	18	2.6	5.7	3.6 »
144—173	9	2	11	2.6	1.3	2.2 »
>173	11	2	13	3.2	1.2	2.6 »
Total	341	159	500			100 »

may be estimated at ± 10—15 per cent, depending on the preparation.

The hydroxytyramine normally present in urine is not sufficient to influence the results of the biological assay to any considerable extent.

RESULTS

Table I and Fig. 1 give the estimated noradrenaline excretion in the total material of 500 cases of hypertension. The figures have been arranged in groups with an average excretion of multiples of 0.02 µg per minute and are given separately for female and male patients.

From the table it can be seen that in about 66 per cent of the total material (64 per cent of the women and 70 per cent of the men) the noradrenaline excretion figures were less than 57.6 µg per 24 hours, *i. e.* within the normal range. Excretions in the range of 57.6—86.4 µg have not been regarded as significantly increased. Since this group represents 17.4 per cent of the material there remains a group of 82 patients, or 16.4 per cent of the entire material, in which the noradrenaline secretion was definitely increa (> 86.4 µg per 24 hours).

The proportion of hypersecretors appe to be fairly constant, for in the five gro of 100 patients each, taken consecutively their samples were analysed, the percent figures were 13, 17, 25, 16, and 11 resp tively.

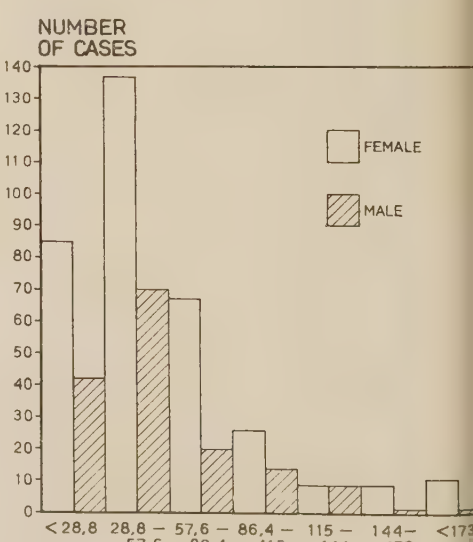

Fig. 1.

ble II. *Distribution of noradrenaline excretion figures in μg per 24 hours according to sex and age.*

	< 28.8		28.8—57.6		57.6—86.4		86.4—115		115—144		144—173		> 173				Total
	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	
.	1		1	1											2	1	3
.	2	5	6	3	3	1		2							11	11	22
.	7	9	8	14	3	5	1	1	1						20	29	49
.	14	8	37	10	8	2	2	3	3		2		2		68	23	91
.	25	6	37	23	22	4	6	1	2	3			5		97	37	134
.	24	12	28	13	21	3	11	4	1	3	4	1	3	1	92	37	129
.	10	2	17	4	10	3	4	2	2	2	2		1	1	46	14	60
	2			2		2	2	1		1	1	1			5	7	12
	85	42	134	70	67	20	26	14	9	9	9	2	11	2	341	159	500
	Normal						Hypersecretion										

noteworthy that except for the excretion ranges (above 144 μg per e figures are fairly proportional be- emale and male patients.

relation of age and sex to the excre- ures is illustrated in Table II where erial has been arranged in age groups ear intervals. The table shows that ypertensives in the present material rage ratio of about 2:1 for females les does not hold for the ages up to s where the males are in excess. r, very few high excretion figures hin this group.

DISCUSSION

esults of the present investigation are d with those of Goldenberg (1951) and 953) who reported that the urinary n of noradrenaline in the majority of essential hypertension was within limits. This is at variance with the of Holtz, Credner & Kroneberg who usually found an increased n in hypertensives. The percentage of hypersecretors in our series is not large (about 16 per cent) but may nevertheless be of significance. The occurrence of an increased noradrenaline excretion, and presumably production, in essential hypertensives suggests that this factor may be of significance in the pathogenesis in a certain proportion of such cases.

The hypersecretion in the 16 per cent showing the highest noradrenaline output may be compared with the figures obtained during infusion of noradrenaline. An additional urinary output of 0.20—0.30 μg noradrenaline per minute was obtained during infusion of 0.12—0.16 μg noradrenaline per kg per minute with a concomitant increase of the blood pressure of 30—40 mm Hg. Although an average output of 0.10 μg noradrenaline per minute as found in some of our hypertensives (144 μg per 24 hours) corresponds to a noradrenaline infusion which would only raise the blood pressure about 10—20 mm Hg, an infusion of noradrenaline may not be strictly comparable to a release of this substance at vasomotor

nerve endings in regard to its pressor effectiveness. Thus an excretion figure of 0.10 μg per min. or even lower, may correspond to a transmitter release capable of raising the blood pressure to a much greater extent. Before more is known about this relationship the true significance of the increased excretion in some cases of essential hypertension cannot be assessed, but the possible relations of such hypersecretion to hypertension of neurogenic or vasomotor origin should be considered. The agreement betwen the percentage of hypersecretors in our series of hypertensive patients and the percentage showing a favorable response in blood pressure after sympathectomy (Corcoran, Page, Taylor & Dustan, 1951) may be merely fortuitous but is suggestive of a causative relationship.

It may be assumed for several reasons that the increased secretion of noradrenaline observed in some of our cases of hypertension is derived from the adrenergic nerves, in particular the vasomotor nerves. Although the suprarenals in man contain some noradrenaline, the amount is small (on an average 0.09 mg per g, Euler, Franksson & Hellström, 1954 a), and the excretion is little changed after adrenalectomy (Euler, Franksson & Hellström, 1954 b).

SUMMARY

1. In 500 unselected cases of "essential" hypertension consisting of 341 females and 159 males, the noradrenaline secretion in urine was within normal limits in 66.2 per cent, (less than 57.6 μg per 24 hours) and increased in 16.4 per cent of the cases (above 86.4 μg per 24 hours).

2. The proportion of males was hig in the age groups up to 30 years than in age groups 30—70 years.

3. In the groups with the highest exc tion (above 144 μg per 24 hours) 20 w females and 4 males.

The possible relation of noradrenal hypersecretion to the pathogenesis of called neurogenic hypertension is discuss

ACKNOWLEDGMENT

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EVALUATION OF AN ACETIC ACID — SULPHURIC ACID METHOD FOR QUANTITATIVE DETERMINATION OF CHOLESTEROL IN SERUM

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New methods for quantitative determinations of cholesterol in serum are frequently suggested. Schoenheimer & Sperry's (1934) and Bloor's (1922) methods are probably the most commonly employed at present but they are both complicated. In 1949 Kingsley & Schaffert suggested a very simple method, but the cholesterol esters were not hydrolysed and these give a stronger color with Liebermann-Burchard's reagent than does free cholesterol. The authors employ a factor of 0.84 in an attempt to make a correction for this, but as the ratio between free cholesterol and the ester compounds is not constant, especially under pathological conditions, this method does not always give the same values as Schoenheimer and Sperry's method.

A very simple method was recently reported by Zlatkis, Zak & Boyle (1953): 0.1 ml serum is pipetted off into 3 ml glacial acetic acid in a large test tube and under this are carefully added 2 ml concentrated H_2SO_4 containing 2 mg $FeCl_3$. The two layers are now mixed rapidly and the clear purple solution is cooled to room temperature and recorded in a spectrophotometer at 560 mμ. The authors state that free cholesterol and cholesterol esters developed color with equal speed and of equal intensity. The experiments were performed with cholesterol tate. The glacial acetic acid which t authors used gave no reaction with try phan.

An equally simple reaction for cholest was employed as early as 1898 by Lifsch He dissolved pure cholesterol in glacial ac acid and added various oxidizing age $FeCl_3$, chromic acid, $KMnO_4$, benzoyl oxide, followed by a few drops of concentr H_2SO_4 and obtained "beautiful colo Later (1907) he demonstrated three st by means of careful oxidation: the oxidation stage gave a red-violet color w gradually became blue, the second ga pure green color and the third gave — nor Liebermann's reaction — in con to the first two stages. The first oxida stage gave absorbtion in the yellow-g part of the spectrum (corresponding to a 560 mμ) and the second stage in the part of the spectrum (corresponding to a 640 mμ). With benzoyl peroxide and bo once or twice, most of the cholesterol oxidized to the first stage, but some remained unchanged. If one drop of F was added, oxidation to the second stage place immediately, and with one dro 10 per cent chromic acid it progressed ther to the third stage. Ester compo

xtremely resistant to the oxidizing and the higher fatty acids interfered e reaction.

hütz used this reaction only for quali- letermination of the various stages ation and he believed that there was holesterol in the first stage of oxida- esent in the serum. In the reaction de- by Zlatkis *et al.*, the same reagents are ut much larger quantities of H_2SO_4 s is added at the stame time as the g agent, $FeCl_3$. To investigate r this reaction is suitable for quanti- eterminations of cholesterol in serum be ascertained that all of the chol- is oxidized to the first stage but not — and that the sample for a blank s not contain any pre-oxidized chol- or that it might lead to incorrect rol values due to the absorbtion of ubstances at 560 mμ. The following ort of the results of such experiments ure cholesterol solutions and with

EXPERIMENTS WITH PURE CHOLESTEROL

etermine whether slight inaccuracies ting the viscous concentrated H_2SO_4 ne analyses would lead to significant relatively small variations in the es employed were tested. This led iderable variation of the results. As heat developed during the mixing of gents (60—65°), experiments were ed with the test tubes placed in r bath of different temperatures be- ixing. This also led to considerable n in the results for the same standard . The glacial acetic acid we used p. a.) gave a marked glyoxylic acid reaction with tryptophan, and the absorbtion curve for this solution had a maximum around 520 mμ.

In order to record the analysis in an Evelyn photometer (at least 6 ml of fluid) the original procedure requires 2.4 ml H_2SO_4 (= 2.4 mg $FeCl_3$) + 3.6 ml acetic acid. To obtain a suitable extinction we used 0.1 mg cholesterol or 0.05 ml serum.

By mutual adjustment of the quantities of H_2SO_4 and acetic acid (0.1 ml), but constant total volume (6.05 ml), marked variations in the optical density were obtained (Table I, experiment 1). When the amount of $FeCl_3$ was doubled, the optical density was definitely increased (experiment 2).

The test tubes with the analyses were placed in water baths of different temperatures before the H_2SO_4 and the acetic acid were mixed. This also led to a marked difference in the results (experiment 5). Freshly made or two days old mixtures of $FeCl_3$ and H_2SO_4 gave no significant difference (experiment 6).

Altering the quantity of H_2SO_4 had the greatest influence on the results (experiment 7). When the amount of H_2SO_4 is kept constant (3 ml), small variations in the amount of $FeCl_3$ caused no difference (experiment 4), while large amounts (3—12 mg) gave uneven results (experiment 8).

The difference between 3 and 6 mg $FeCl_3$ when the analyses are mixed after heating to 100° (experiment 8), was slight in contrast to when the analyses were left standing in air (experiments 1 and 2). It is also seen that the results of the analyses vary from day to day, even when the experimental conditions are kept constant. A standard curve set up according to the original directions and recorded in Beckmann's spectrophotometer is also unsatisfactory.

EXPERIMENTS WITH SERUM

In the experiments with serum the original method was used and the results read in a Beckmann spectrophotometer. Here too there was a marked difference in the results if the amount of H_2SO_4 was altered by 0.1 ml. There was a pronounced increase at 560 mμ

Table I. *Cholesterol, 0.1 mg in 6.05 ml total volume recorded in an Evelyn photometer.*

Experiment No.	Quantities		Opt. Density Filter 565	Before mixing, test tube kept in
	H_2SO_4 ml	$FeCl_3$ mg		
1.	2.6	2.6	0.112	Air
	2.7	2.7	0.248	»
	2.8	2.8	0.288	»
	2.9	2.9	0.244	»
	3.0	3.0	0.240	»
2.	2.6	5.2	0.420	»
	3.0	6.0	0.377	»
3.	Constant	Constant		
	2.8	2.8	0.288	Air, mixed rapidly
	»	»	0.268	» » »
	»	»	0.272	» » slowly
5.	»	»	0.220	Water bath: ice water
	»	»	0.270	» » 20°
	»	»	0.280	» » 40°
	»	»	0.319	» » 60°
	»	»	0.347	» » 100°
6.	»	»	0.320	Water bath: 100° Freshly mixed H_2SO_4—$FeCl_3$
	»	»	0.319	Water bath: 100° Freshly mixed H_2SO_4—$FeCl_3$
	»	»	0.328	Water bath: 100° 2 day old mixture
7.	Varied	Constant		
	2.6	2.8	0.320	» » »
	2.8	2.8	0.328	» » »
	3.0	2.8	0.305	Wather bath: 100°
4.	Constant	Varied		
	3.0	2.6	0.231	Air
	»	2.8	0.224	»
	»	3.0	0.231	»
8.	»	3.0	0.469	Water bath: 100°
	»	6.0	0.438	» » »
	»	8.0	0.372	» » »
	»	10.0	0.392	» » »
	»	12.0	0.055	» » »

There is a considerable difference in the optical density on alterations of the quantities of H_2SO_4 o $FeCl_3$ or both, or with varied temperature before mixing the reagents.

when the analyses was preheated in a boiling water bath as compared to a water bath at room temperature. But when absorbtion curves from 500—620 mμ were drawn up for these two analyses, the peak at 560 m was almost hidden in the heated analys (Fig. 1, I) — other colored compounds we formed simultaneously. The curve for t

ed analysis was considerably lower, had a definite peak around 560mμ , II and Table II, serum 1). The ion curve reported by Zlatkis *et al.* is very similar to the curve for the analysis.

here was such a big difference between wo curves, attempts were made to get till more of the interfering substances ling the analysis in an ice bath. rent serum was used for these ana- ne of which was performed as above ter bath at 20°, the other in an ice These two curves were practically (Fig. 2, I and II) and were similar curve for serum No. 1 at 20°. But ling at 560 mμ was definitely lower analysis carried out in the ice bath ble II). There was thus a big dif- in the readings at 560 mμ dependent temperature at which the reaction ace. On rapid mixture in air as de- in the original method, the tempera- he analysis rose to 60—65°.

etermine whether it was possible to um + reagents *without* $FeCl_3$ as a his was done at the same time as the nentioned analyses. In the heated

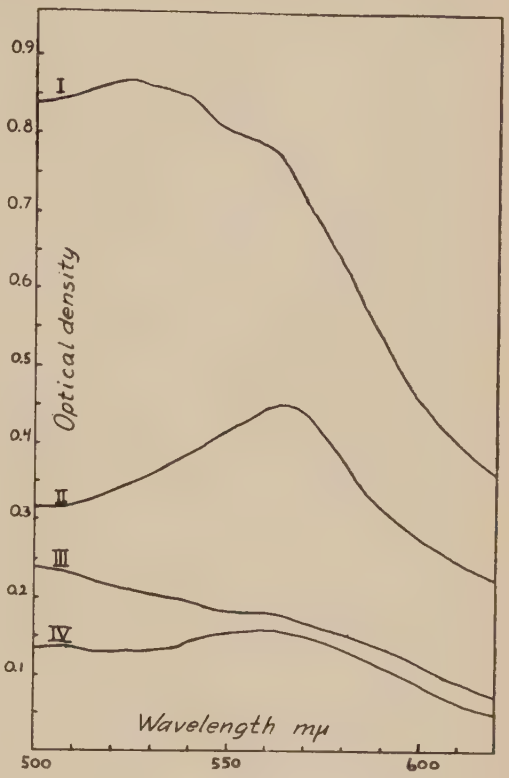

Fig. 1. Absorbtion curve for serum No. 1, analysis and blank in water bath at 100° (I and III respectively) and at 20° (II and IV respectively).

"blank" the optical density decreased regularly from 0.24 to 0.08 with a minimum

Table II. *Serum, 0.05 ml in 5.05 ml total volume, recorded in a Beckmann spectrophotometer.*

		Analysis		Blank	
		520 mμ	560 mμ	520 mμ	560 mμ
Serum No. 1	100°.....	0.860	0.783	0.212	0.177
	20°.....	0.330	0.443	0.127	0.155
Serum No. 2	20°.....	0.280	0.352	0.082	0.108
	0°.....	0.185	0.260	0.095	0.119

There is a considerable difference in the optical density dependent on the temperature before mixing the reagents.

Fig. 2. Absorbtion curve for serum No. 2, analysis and blank in water bath at 20° (I and III respectively) and at 0° (II and IV respectively).

irregularity at 560 mμ (see Fig. 1, III and Table II, serum No. 1). In the "blank" performed at room temperature the curve was markedly lower but almost horizontal with a small peak around 560 mμ (Fig. 1, IV). The values recorded at 560 mμ were close together (Table II).

The corresponding "blank" for serum No. 2 were carried out in water baths at 20° and in ice water. The curves were practically identical (Fig. 2, III and IV) and were similar to the curve for serum No. 1 at 20°. The "blank" was thus little influenced by temperature but there was a big difference between the two sera. This shows that a blank with reagents only may lead to error as this difference is not compensated. On the other hand, there was a slight peak around 560 mμ which may indicate that cholesterol has some effect in this type of "blank" so this will not be correct either.

With the many uncertainties involved in this method, we have come to the conclusion that in its present form it is unsuitable routine determinations of cholesterol serum.

SUMMARY

Small variations in the amount of visc concentrated H_2SO_4 added (0.1 ml) changes in temperature during the mixt of the former with glacial acetic acid led pronounced differences in the results of analyses. It was difficult to obtain unifo readings from day to day, even with p cholesterol solutions.

Serum must be included in the blank as different sera have different absorbt curves, between 500 and 600 mμ. But it d not seem that serum + reagents *without* oxidizing agent can be used as a blank, such serum analyses have a slight absorbt peak at 560 mμ which may be due to oxidation of some cholesterol also here.

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THE MEASUREMENT OF DEUTERIUM OXIDE AT LOW CONCENTRATIONS BY THE GRADIENT TUBE METHOD

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cent years heavy water, deuterium as found increasing use as an im- tool in clinical studies of total body water metabolism and body com-

ch experiments an amount of heavy usually injected to give an equi- value in the body fluids of 0.100 to ol. per cent, and in order to get results this low concentration must rmined with an accuracy of 1 to 2

high degree of accuracy is obtainable alling drop method and by the mass neter (Schloerb, Friis-Hansen, Edel- heldon & Moore 1951 and Solomon, n & Soloway 1950). However, both are rather elaborate, whereas the tubes of Linderström-Lang offer e method for the determination of vater over a wide range of concen- (Linderström-Lang & Lantz 1938 derström-Lang, Jacobsen & Johan- 3).

paper will present a brief description method and the accuracy which is le at low concentrations of heavy n the hope that investigators who use heavy water in their studies l this a feasible method well within the limits of their laboratory facilities. Some practical aspects of the method are given here. The original description by Linderström-Lang includes a theoretical discussion to which the reader is referred.

A short description has also been given by Arnfinsen 1946.

THE GRADIENT TUBE

When two liquids of different densities and soluble in each other are layered, the lighter on top of the heavier, there will be a gradual change in density in the mixing zone. This fact was used by Linderström-Lang for accurate density determinations in the gradient tube, which (Fig. 1) consists of a vertical glass tube connecting two glass bulbs. It is filled with two different mixtures of kerosene and bromobenzene, the lighter on top of the other, after which a gentle mixing in the connecting tube is carried out with a glass spatula. The gradient tube is placed in a constant temperature bath with a glass front.

When a drop of water containing heavy water is introduced in the top of the gradient it will sink untill it reaches a layer with the same density as its own. The density of the drop, or the concentration of heavy water, is then calculated by comparing the position

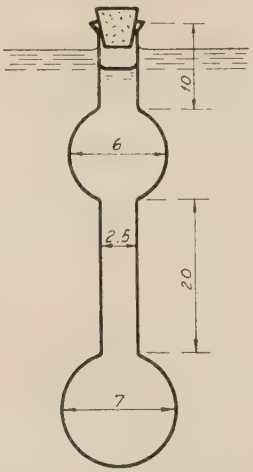

Fig. 1. The gradient tube.

of the drop with that of standard drops with known concentration of heavy water.

In this study the purification of plasma-water and the preparation of standards was carried out as previously described (Schloerb, Friis-Hansen, Edelman, Sheldon & Moore 1951). The sensitivity of the method depends on 5 points:

1. The density difference between the two kerosene/bromobenzene mixtures should be small in order to obtain a small variation of density per unit of distance.
2. The change in density per unit vertical distance should be constant, at least in the middle third of the connecting tube. Standard drops with the same difference in concentration must orient themselves at equal distances in height, and several drops of equal concentration must stay in the same horizontal plane.
3. The drop size must be equal.
4. The position of the drops has to be measured with great accuracy. In order to obtain this it is necessary to us cathetometer microscope.
5. The temperature control must be hig sensitive.

1) In the original description of method the densities of the two mixtu were given as 0.990 and 1.021. This give a working range of 0—11 per heavy water, but in most clinical studies concentrations which one encounters from 0.0 to 0.4 vol. per cent. For this pose mixtures of a density of 0.998 and 1. were found to be the most suitable. Wit bath temperature og 25° these will giv gradient in which a difference of 0.100 per cent corresponds to a difference in he of 10 mm.

These mixtures are prepared in the lowing way: 300 ml of bromobenzene (sp 1.49) are added to 730 ml of kerosene (sp 0.79). A liquid of a density of 1.00 at r temperature is hereby obtained. This den is measured accurately and the final adj ment is carried out by adding small amo of bromobenzene or kerosene. Rou speaking 1 ml of the first added to 500 of the mixture will increase the density 0.001 and 1 ml of kerosene will make liquid lighter by 0.0004.

The density determination in this lab tory is carried out as follows:

Two glass-stoppered weighing bottles equilibrated on a balance and 5.000 ± 0 ml of the liquid is transferred into one o bottles by a Krogh-syringe (Krogh, 19 The weight of this volume is recorded t mg, and 5 ml is then transferred into other bottle. After 10 such determinat the density at the given room tempera

ılated with an accuracy of more than

the mixtures are shaken three times
ual volumes of distilled water. The
ion is facilitated by placing the flask
rigerator, after which the liquids are
or 48 hours by shaking with "Sili-
After filtering the liquids are brought
temperature of the bath, and the
is poured into the gradient tube up
middle of the connecting tube. The
s then carefully allowed to flow down
glass rod on top of the other up to
h below the water surface level.
is then completed by rotating a glass
with up- and downward strokes from
dle to the ends of the connecting tube
seconds. One to two days later the
ready for use.

he gradient is tried out by inserting
mm³ drops of each of the standards
0.050 — 0.100 — 0.150 — 0.200 and
l. per cent D_2O. The drops should
ent themselves in horizontal layers
intervals. Often this is not the case
e tube is tested for the first time,
r a couple of experiments it will
become satisfactory. This improve-
presumably achieved because the
layers become saturated with water
ng heavy water at the different con-
ns. If the drops do not find their
um in the middle section of the con-
tube, this may be corrected by chang-
r the bath temperature or the density
ures.

rops are removed by a thin glass rod
small piece of hardened filter paper
around the end, and 24—48 hours
tube is ready for a new determina-
tion. The tube is carefully closed to prevent evaporation and contamination of the surface.

Sometimes the drops tend to rise or sink slowly rather than reaching a stable position. This may be caused by convection or by water soluble impurities in the mixtures. In the latter case renewed rinsing with distilled water and subsequent drying will remove this source of error.

3) A drop size of 1 to 3 mm³ may be used. The absolute volume of the drop is not so important, but all drops must be of equal size. This is best achieved by using a Lang-Levy pipette. The bigger drops will sink faster through the gradient and reach their resting position more rapidly, but when several drops are used, all within the same range of concentration, it is important that the drops are small enough that they do not

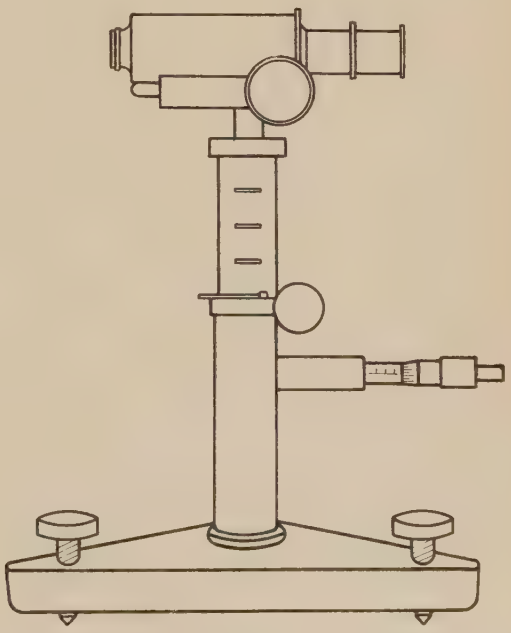

Fig. 2. The cathetometer microscope.

interfere with each other. On the other hand any contamination will introduce a greater error the smaller the drop size.

The drops are blown out under the surface of the kerosene mixture and will not leave the pipette until this is gently lifted out. It is of great importance that the surface be free from any contamination. Therefore the gradient tube must be kept well stoppered.

When the drops are introduced into the gradient they will require half an hour to one hour before achieving equilibrium and the reading should not be made before this has taken place as the heavier drops will reach their end point before the lighter ones.

4) The exact position of the drops is measured by a cathetometer microscope [1] (Fig. 2), which has free movements in the horizontal plane and where the height position of the microscope is read on a micrometer screw built in on a side arm to 0.01 mm.

5) The temperature control of the bath is of greatest importance. A thermoregulator as shown in Fig. 3 has been used. It consists of a thin-walled tube filled with ether, as this has a very high thermal expansion coefficient. The vertical thick-walled tube is filled with mercury up into the capillary section. The side arm *a* is for filling, and *b* is the negative electrode melted into the wall. The positive electrode is a platinum needle on the end of a brass rod cemented to the glass cap *c*.

A low ampere electric current regulates the heating current through a magnetic contact. The heating is carried out by an electric

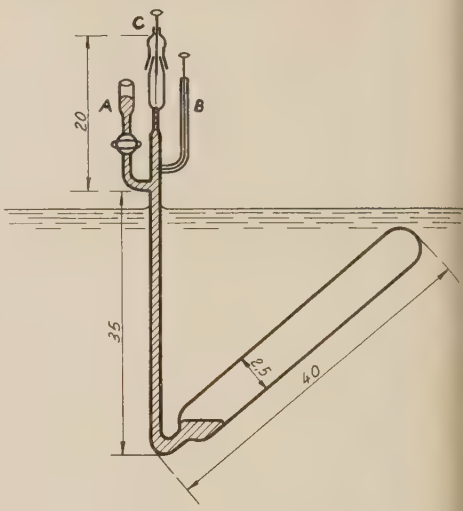

Fig. 3. The thermoregulator.

coal-thread bulb which has a very short lat period.

The cooling of the bath is due to e poration from the surface of the water wh is stirred vigorously by a motor. If the re temperature rises above that of the ba cooling is effectuated either by a control flow of cold water through a copper spi or by letting a fan blow on the surface. W regulating the heating against the cool these two factors should balance in such way that they are of equal length of t and so that switching takes place every 5 15 seconds.

The importance of an accurate tempe ture control is shown by the observation t a change in temperature of 0.001° co sponds to 0.05 mm movement of the dr

CALCULATIONS

When total body water determinations carried out, an amount of heavy wate injected to give an equilibrium concentra

[1] Constructed by and obtainable from: Dich, Brøndby Strand, Denmark.

0.100 and 0.200 vol. per cent. This
nined by using standards of 0.000,
d 0.200 vol. per cent. The unknown
will then orient themselves between
ier standards, and by comparing the
between the standard drops the
of the gradient is checked, and the
everal drops of the same concentra-
show whether or not a given layer
ntal. The unknown is calculated by
nterpolation.

ACCURACY

erence of 0.100 vol. per cent corre-
to about 10 mm, which may be
d to 0.01 mm giving a theoretical
of 0.0001 vol. per cent, but due to
hs in the linearity and in the hori-
lane this high degree of accuracy is
ined.

consecutive experiments the 0.100
s were determined from the position
.000 and the 0.200 standards. The
as: 0.1008 ± 0.0008 vol. per cent,
an accuracy of 2 per cent or 1 per
the interval between the standards.
r to obtain this accuracy it is ne-
to use the tube frequently. After a
f rest the results will be less satis-

total body water is measured, blood
are drawn two and three hours after
and distilled separately. Both samples are determined in two gradient tubes, whereby the total body water calculation is based upon the average of the four values.

SUMMARY

A brief description is given of the measurement of heavy water by the gradient tube, which offers a simple and accurate method for determinations at low concentrations.

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THE ELECTROPHORETIC MOBILITY OF CHYLOMICRO IN A CASE OF ESSENTIAL HYPERLIPEMIA

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(Received for publication Nov. 17, 1953)

If the lipids of serum are present as particles greater than ¼ of the wave length of visible light[1] serum becomes turbid. As suggested by Gage & Fish 1924 these lipid particles are called chylomicrons.

This visible lipemia is normally met with in the absorptive state after a fatty meal. It also occurs in cases of disturbed lipid metabolism, *e. g.* nephrosis, hypothyreosis, diabetes and essential hyperlipemia. The pathogenesis of this abnormal chylomicronemia is as yet not completely understood. Possible mechanisms are disturbed phospholipid metabolism (Ahrens & Kunkel, 1949) and/or deficiency of some of the components of the "clearing system" (Brown, Boyle & Anfinsen, 1953).

Investigations of the electrophoretic mobility of chylomicrons in serum by means of free electrophoresis have shown cloudiness in the α- and β-globulin regions, indicating association of the chylomicrons with the α- and β-globulins (Blix, 1941, McFarlane, 1949).

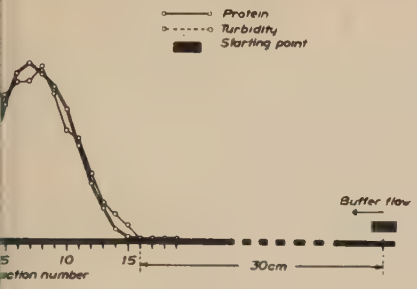
Swahn (1953) has shown by free electrophoresis before and after high speed centrifugation that greater chylomicrons ($> 0.5\ \mu$) migrate with the α-globulin fraction, w smaller chylomicrons ($<0.5\ \mu$, "lipomicron cloud the β-globulin region. In a case essential hyperlipemia he found chylomicr associated with the α- as well as the globulins.

The latter author also studied the beha of milky hyperlipemic serum in paper elec phoresis, but paper was found to be uns able as a supporting medium, above all to adsorption of β-lipoprotein and ch microns to the paper. After Kunkel & Sl (1952) pointed out that starch does not sorb lipoproteins we have found it to be useful supporting medium for the separa of the serum lipoproteins. A study of electrophoretic migration of chylomicron starch medium was considered to b interest as this has not been investig earlier. A correspondance between the trophoretic mobilities in free electropho and zone electrophoresis in starch woul a strong indication against adsorptio chylomicrons in the latter medium.

METHOD

The electrophoresis is carried out *vertical* tube filled with starch. When trophoresis is completed, air pressur

[1] (*i. e.* $> 0.1\ \mu$.)



Migration of hyperlipemic milky serum
he starch column by means of simple
buffer-flow.

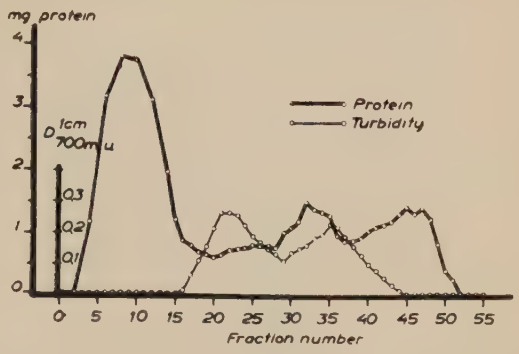


Fig. 2. Electrophoretic separation of the hyperlipemic serum in starch medium. Diagram showing the relative mobilities of the chylomicrons and the protein components.

) the cathodic end of the tube. The
proteins are forced out by the
w through the anodic end of the
are collected in fractions of suitable
A complete description of the
s in press (Carlson, 1954).
ertain that there is no adsorption
rch, a certain amount of the serum
vestigated was forced through the
imple buffer-flow and the fractions
for protein and visible lipids.

RESULTS

from a patient with essential hyper-
total lipids 4060 mg per cent, chol-
7 mg per cent, phospholipids 673 mg
was subjected to starch electro-
as described above. The fractions
lysed for protein according to a
Folin-Cicolteau method (Lowry,
ugh, Farr & Randall, 1951). The
of the fractions was read at 700 mμ
man spectrophotometer.
sults of buffer-flow are shown in
ich indicates that there is no signi-
orption of the chylomicrons to the

The result of the electrophoretic separation is demonstrated in Fig. 2. It is seen that the chylomicrons migrate as two distinct fractions, one associated with the α-globulin and the other with the β-globulin. This finding is in good agreement with the above-mentioned observations in free electrophoresis. The diagram also shows the distribution of the chylomicrons in respect to the serum proteins. However, as the chylomicrons in the α- and β-globulin region are of different size, the density values give no information of the real quantitative distribution. The present data clearly demonstrate the superiority of the starch method compared with the zone electrophoresis in paper, which gives the erroneous impression of a single immobile chylomicron fraction (cf. Swahn, 1953).

Fig. 3 is a photograph of the fractions collected after electrophoresis, which shows that the turbidity appears in fraction 17 and disappears in fraction 44.

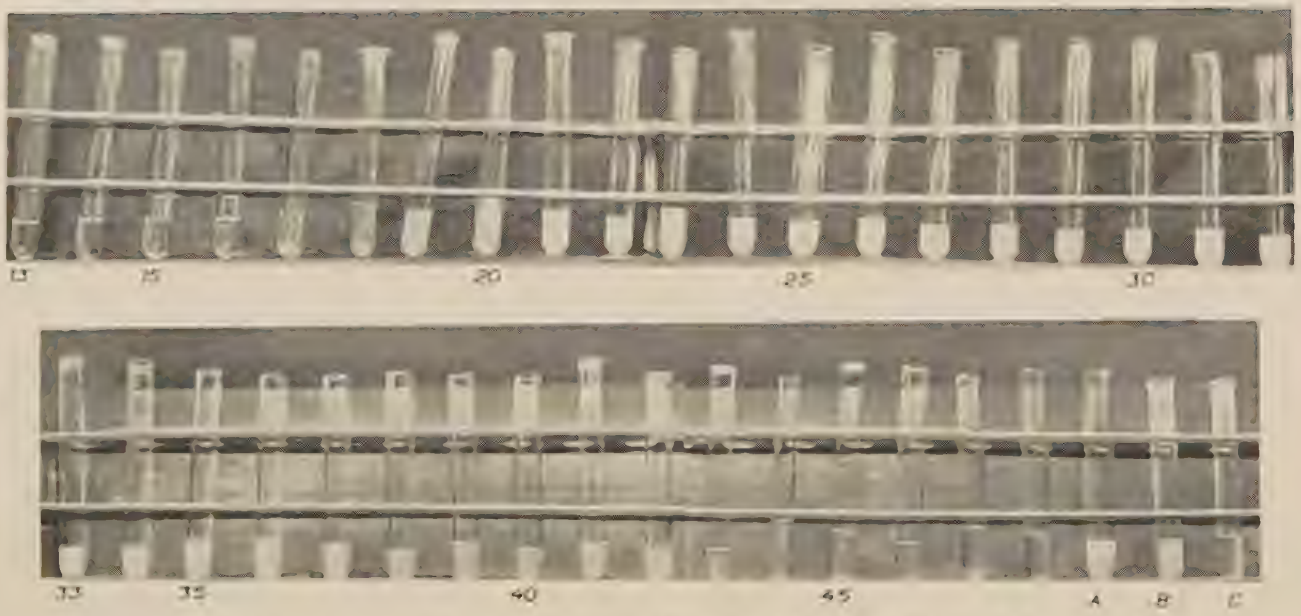


Fig. 3. Turbidity of some of the separated serum fractions (cf. Fig. 2). Numbers = fraction number. A = undiluted serum. B = serum diluted 1 : 10. C = serum diluted 1 : 100.

SUMMARY

The electrophoretic mobilities and the istribution of chylomicrons in serum have een demonstrated by means of a modificated one electrophoresis technique in starch ıedium. The results also indicate that there s no appreciable adsorption of chylomicrons ı starch.

Further investigations are in progress and vill be published later.

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A NEW TIME-SAVING APPARATUS FOR PAPER ELECTROPHORESIS

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(Received for publication Aug. 22, 1953)

In recent years the quantitative fractionation of serum proteins has become a method of routine use in the clinical laboratory. Many improvements of the technique have been attempted but nevertheless the method is still comparatively slow. In most apparatuses fraction-times of 9 to 16 hours are necessary which often means that two different people have to handle the analysis. The calculation of the proportion between the concentrations of the fractions requires the drawing of an absorption curve and its planimetric treatment.

In the apparatus described here the fractionation time is only 3 to 5 hours but nevertheless the protein fractions are so sharply separated that they can be cut apart and no absorption curve is needed. The apparatus was built by us in the laboratory workshop.

The new apparatus is made of transparent plexiglass (Bonoplex from A. B. Bofors Nobelkrut, Tidaholm, Sweden) and is constructed to keep the papers in a moist chamber with watersaturated air. The papers are suspended in such a way that they rest horizontally on a number of sharp nylon needles and their surface * are not in contact with any other surface. In this way diffusion and convection currents are avoided.

The apparatus consists of 5 different parts (Fig. 1): the bottom plate (1), 2 electrode vessels (2), the paper bed (3) and a tight fitting hood (4). The bottom plate is provided with a number of erecting guiding pins (5) keeping the electrode vessels in a fixed position. For its fixation the paper bed too is provided with guiding pins (6) preventing the hood being pressed down to the paper. The bottom plate has a contact at one of the short sides for connection with the source of 220 V DC. Each electrode vessel is built to contain 250 ml buffer solution. By an inner wall parallel to the long outer walls they are divided into two communicating parts, one for the electrode, and the other for the ends of the papers. The electrodes are platinum wires, about 30 cm long, fixed in the vessels. The paper bed is rectangular 15 × 32 cm. Its top side is covered by thin

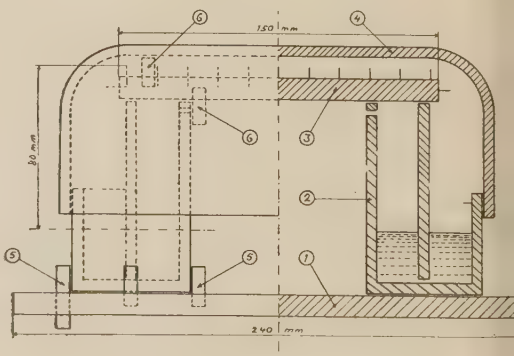

Fig. 1.

* It ought to be mentioned that before our apparatus was constructed the company LKB-Produkter Fabriks A.B., Stockholm, had demonstrated an apparatus in which the paper rested on polystyrene cones.

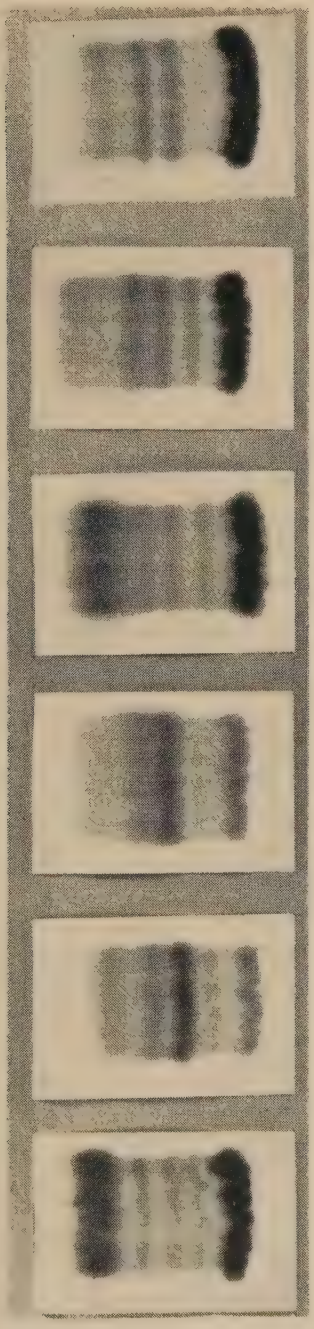

Fig. 2.

nylon brush bristles about 10 mm long of which about 5 mm protrudes from bored holes in the bed, narrow enough to keep the bristles in a very fixed position, thus forming a "fakir bed". The distance between the bristles is about 15 mm. The hood fits tightly on the sides of the bed and on the outsides of the electrode vessels thus forming a closed room, the air of which is nearly saturated by moisture from the electrode vessels.

The size of the apparatus can be varied within wide limits but with the size described here 6 electrophoresis experiments can run simultaneously. The apparatus can be provided with Volt- and Ampere-meters, timed off-switching, signal-lamps etc. but this outfit is unnecessary in routine work.

Paper strips (Whatman No. 1) of about 40 mm × 320 mm are used. The serum is applied by an Ellerman pipette. The site of application should be so chosen that it comes about 5 cm from the edge of the paper bed. After application of the serum the strips are moistened with the buffer solution. The free ends of the paper dip into the outer compartments of the electrode vessels. These are filled with a 0.1 molar barbituric acid buffer solution pH 8.6. The current used is 220 V DC. With 6 paper bands in the apparatus the current strength varies between 14 and 17 mA. The electrophoresis has to run 3 to 5 hours.

The protein fractions are stained with brom-phenol-blue or Ponceau 2R in the usual way and come out so sharply that they can be separated by cutting out each fraction. In Fig. 2 some uncut strips are demonstrated. They are from experiments run for 4 hours. The sharp separation of the protein fractions cannot be demonstrated as clearly on a photograph as they are on the original strips. The two top pictures are from healthy human subjects. In all the pictures the serum had been applied to the left, the thick band to the right representing the albumin fraction.

A POWER SOURCE FOR FILTER PAPER ELECTROPHORESI

By M. U. TSAO

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(Received for publication Oct. 10, 1953)

During the past few years considerable interest has developed in the applications of filter paper electrophoresis. All the apparatus described thus far requires manual control of electric potential in order to keep the current constant. (Durrum, 1950; Cremer & Tiselius, 1950.) For reproducible results and possible calculation of the mobility rate of proteins the maintenance of a constant current through the filter paper seems desirable. According to Durrum a direct relationship of migration rate and electric field strength may be postulated and the field strength may be expressed as follows:

Field strength = Current × Cross-sectional area^{-1} × k^{-1} where k is the conductivity of buffer solution at that particular cross section. A constant field strength can be obtained when k value as well as currents is held constant. This is true when the buffer concentration change due to evaporation is prevented (by sandwiching the paper between two sheets of plate glass, by immersion in an immiscible solvent, or by low temperature) and the current is maintained constant.

This note describes the adaptation of a power source, which has been in use in this laboratory for the determination of plasma or serum total base by electrodialysis, to filter paper electrophoresis with a minor modification. (Tsao, 1950.) Simultaneous runs of electrodialysis and filter paper electrophoresis may be carried out with independent control of current for each proces The detailed electronic circuit of this pow source has been presented by the author i an earlier communication; hence only th modification will be reported.

For the purpose of electrodialysis it wa found that a pulsating direct current wa sufficient to achieve the complete separatio of cations; therefore, no particular effo was made to smooth out the rectified alte nating current power supply. In order t furnish smoothed-out direct current fo electrophoresis two large capacitors (1 microfarad, 700 working voltage) were so dered across the output of the rectifier tub 5R4GY (or 5Y3), in the arrangement wit the 15 Henry inductance in the middle.

With this simple modification the app ratus is capable of furnishing a potential t to 350 V and automatically regulated curre strength from 0.5 MA to 5 MA or great depending on the total resistance of the ele trophoresis system. Since the apparatus ma be constructed to have a number of indepe dently controlled direct current sources, o can run several filter paper electrophores at different current strengths and at the sam time do a number of electrodialyses for tot base determination. Once the desired curre strength has been set at the start of th respective processes by the adjustment the 5000 ohm variable resistor, no mo supervision is required until the desir

ngth of time has elapsed. Satisfactory sults have been obtained repeatedly in this oratory when one electrophoresis and five ctrodialyses were carried out.

SUMMARY

A power source for filter paper electrophoresis with several outlets for independent and automatically controlled current engths has been presented.

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DETECTION OF ADRENALINE AND NORADRENALINE ON PAPER CHROMATOGRAMS

Most of the color reactions for the paper chromatographic detection of adrenaline and noradrenaline are based on the oxidation of these substances to colored compounds by common oxidative agents (e. g. James 1948; Shepherd & West 1951). The oxidation includes the formation of the corresponding quinone and an indole ring closure. The smallest amounts that can be detected by these reactions are, as a rule, approximately 1—2 μg of adrenaline or noradrenaline.

The sensitivity of these reactions can be greatly improved by spraying the paper after the oxidation with a strong, acidic and alcoholic solution of p-dimethylaminobenzaldehyde, which reacts with the indole ring containing oxidation products of adrenaline and noradrenaline. Of the various oxidative agents tested, a weak solution of potassium ferricyanide and a weak solution of iodine proved the most suitable. When a solution of 0.2 per cent potassium ferricyanide is used as the oxidant, p-dimethylaminobenzaldehyde produces an intense blue color with adrenaline, noradrenaline being first transformed into a yellow compound, with a strong, yellow-brown fluorescence which gradually fades as the blue color develops. When the oxidation is performed with 0.001-n iodine in 0.2-M acetate buffer solution at pH 6 and the solution of p-dimethylaminobenzaldehyd is sprayed after an interval of 3—5 min., the blue color develops immediately with both adrenaline and noradrenaline. Adrenaline and noradrenaline can be differentiated if the iodine oxidation is carried out even at pH 3.5, when only adrenaline is oxidized.

The limit of sensitivity of this color reaction is approximately 0.2 μg of adrenaline or noradrenaline.

Another type of method can be derived on the basis of the observation that ethylenediamine forms stable and strongly fluorescing compounds with adrenaline and noradrenaline (Natelson, Lugovoy & Pincus 1949). This reaction can also be readily applied with paper chromatograms. The best results are obtained by keeping the paper in ethylenediamine vapor at 100—110° C for half an hour. Adrenaline and noradrenaline are transformed into compounds of a weak brown color with stable, strong yellow-brown and yellow-green fluorescences, respectively. As little as 0.4 μg of adrenaline or noradrenaline can be detected by this method.

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Department of Medical Chemistry,
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Nov. 20, 1953.

Martti-Elja Pitkänen

PAPER CHROMATOGRAPHY OF UROERYTHRIN

In this paper a study of uroerythrin by per chromatography is reported. Uroery- rin is a pigment which is precipitated with e urate sediment and gives it a red color. is very sensitive to light and to oxydizing d reducing substances. The color dis- pears at heating and is changed by treat- nt with strong acids and alkalis. Uroery- in is soluble in amyl alcohol and to some gree in ethyl alcohol, ethyl acetate and oroform. Acid addition increases the ubility.

In an endeavour to develop a paper romatographic method for this substance urines were cooled and in case of a red- h precipitate this was spun off, washed ee times with ice cold water, suspended distilled water and acidified with ⅓ volume 6 per cent acetic acid, after which 1 ml amyl alcohol was added. The precipitate s allowed to pass into the amyl alcohol er and was then centrifuged. The clear, ored amyl alcohol solution was used in paper chromatography.

A number of solutions were tested in order find the best one for uroerythrin chro- tography. Amyl or butyl alcohol buffered h phthalate pH 6.4, and a mixture of butyl-benzyl alcohol and 10 per cent ethanol buffered with phthalate pH 6.4 were rejected as they did not give distinct spots. With butanol—acetic acid—water (40 : 10 : 50) the spot moves in front. Good separation from other substances present was obtained with amyl alcohol—pyridin—water (35 : 35 : 30) but the alkaline reaction was unsuitable for uroerythrin. The best results were obtained with tertiary amyl alcohol—ethanol (40 : 10) saturated with phthalate buffer pH 6.4.

The uroerythrin in amyl alcohol solution was applied on Whatman No. 1 paper previously treated with phthalate buffer pH 6.4. The paper was allowed to stand in the chamber for at least 6 hours and the run was carried out with the buffered tertiary amyl alcohol-ethanol solution.

If large amounts of uroerythrin are present the airdried chromatogram shows a faintly pink spot at Rf 0.66—0.70. Viewed under ultraviolet light the spot possesses an intense orange fluorescence. When treated with concentrated HCl the spot and the fluorescence turned a deep red. Both the color and the fluorescence disappeared on addition of concentrated NH_3.

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ISOLATION OF PYRUVIC AND ALPHA-KETOGLUTARIC ACIDS FROM BLOOD AND TISSUES IN THE PRESENCE OF C^{14}-ACETATE

When existing methods (Cavallini, Frontali & Toschi, 1949 a and b; El Hawary & Thompson, 1953; Hockenhull, Hunter & Herbert, 1953; Seligson & Shapiro, 1952) were used to separate pyruvic and alpha-keto-glutaric acids from biological materials as the 2,4-dinitrophenylhydrazones in the presence of sodium acetate-1-C^{14}, the isolated keto acids were found to be contaminated with labeled acetate. The keto acids were successfully separated from the labeled acetic acid by introducing two new steps: 1. the bisulfite addition complex of the keto acids was formed and the acetic acid extracted from this product with ether and 2. the 2,4-dinitrophenylhydrazones formed from the keto acids derived from step 1 were subjected to vacuum distillation. As a result of these two steps, almost all the contaminating acetic acid was removed and only 0.05 per cent of acetate-1-C^{14} was present in the isolated keto acids. Recoveries of pyruvic and alpha-ketoglutaric acids from blood were 66 per cent. With this procedure the contaminating acetic acid can also be isolated and measurements made of its specific activity.

This method is being used to study the utilization of two-carbon compounds for the synthesis of keto acids.

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HE COAGULANT ACTIVITY OF RUSSEL VIPER VENOM

Russell viper venom has been used in some oratories as a substitute for brain thrombo-stin in "prothrombin time" determina-ns. Its clotting action has been said ently (Biggs, Douglas & Macfarlane, 53) to be similar to that of brain thrombo-stin and to require both proconvertin actor VII) and proaccelerin (Factor V) its full effect. However, we have found t the venom acts independently of the pro-nvertin content of plasma.

Russell viper venom appears to contain the nivalent of the combination of anti-hemo-lic factor A (anti-hemophilic globulin), i-hemophilic factor B (PTC, Christmas tor), and proconvertin. In the presence an optimum amount of platelet-lipoid tor it possesses a clotting activity equi-ent to that of convertin. Thus a 1/10,000 ution of venom in 0.07 gm per cent crude halin suspension will clot normal plasma, mophilia A and hemophilia B plasma, and genital hypoproconvertinemia plasma hin 5 seconds. Like convertin, venom-id is not able to clot proaccelerin deficient sma rapidly.

The P. and P. system can be used to asure prothrombin alone by the substition venom-lipoid prepared as described above brain thromboplastin. Care should be en to remove the last traces of prothrom- from the ox plasma proaccelerin reagent. lues obtained by this technique are com-able to those obtained by the prothrombin thod of Owren & Aas (1951).

A rapid presumptive test for hypoproconvertinemia is to do a Quick prothrombin time determination first with the usual brain thromboplastin and then with Russell viper venom. Proconvertin deficient plasma will give a prolonged clotting time with brain thromboplastin but a normal time with viper venom. Proaccelerin or prothrombin deficient plasma will give a prolonged time with both agents. It is unnecessary to add lipoid to the venom for this test if the blood is centrifuged slowly so that the majority of the platelets remain in the plasma.

Dicumarol and dicumarol like drugs depress both prothrombin and proconvertin formation. If viper venom is used as the "thromboplastin" for clotting time determinations on patients on dicumarol therapy only the depression of the prothrombin content of the plasma will be measured. In the past this has led to serious accidents (Wilson, 1947).

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THE INCREASED COAGUABILITY OF PLASMA EXPOSED TO GLASS

It is well known that contact with a glass surface promotes blood coagulation not only because of the disintegration of platelets but also because of the activation of plasma clotting factors. The latter has been thought due to the removal of an anti-cephalin (Tocantins, 1945), the removal of a prothrombin inhibitor (Fiala, 1951), the activation of prothrombinogen (Quick, 1950) and thromboplastinogen (Quick & Epstein, 1952), and to the activation of Christmas factor (anti-hemophilic B factor) (Biggs, Douglas & Macfarlane, 1953).

We have confirmed the observation of Biggs and co-workers (1953) that glass contact increases anti-hemophilic B factor activity. However, another plasma substance must also be activated for the enhanced plasma coaguability is still demonstrable in the presence of a strong brain thromboplastin which provides the equivalent of the combined effect of platelet factor, anti-hemophilic A factor and antihemophilic B factor. When Russel viper venom is used as the "thromboplastin" the clotting times of "glass" plasma and "silicone" plasma are the same. This suggests that the other substance activated is proconvertin, since the clotting action of viper venom is independent of the proconvertin content of plasma.

The activation of proconvertin by glass can be verified by testing in the specific systems of Owren & Aas (1949) a sample of platelet poor plasma one portion of which is exposed only to silicone coated surfaces and the other portion of which is first shaken with ¼ volume of fine quartz powder fo 15 minutes and the powder then remove by centrifugation. The data from such a experiment are shown below:

Test	Clotting Time (seconds) Silicone plasma	Glass plasm
Quick	13.9	10.4
P. and P.	30.5	24.2
Proconvertin	36.3	28.5
Prothrombin	31.7	31.8
Proaccelerin	29.0	29.7

The increased proconvertin activity reflected in every system except the pr thrombin and proaccelerin systems whe a change in proconvertin content of the te sample is masked by an excess of proco vertin in other reagents. The same resul were found when hemophilia A, hemophil B and parahemophilia plasmas were teste When congenital hypoproconvertinem plasma was tested the Quick time was r duced from 135 seconds to a value of seconds, the P. and P. time from 150 secon to 94 seconds and the proconvertin time fro 107 seconds to 79 seconds due to the activ tion of the small amount of proconvert which this plasma contains. The speci prothrombin time, however, was increas from 28 seconds to 67 seconds for in proco vertin deficient plasma some prothrombin adsorbed into the glass powder. These fi dings emphasize that the increase in coagu bility produced by glass can not be due the activation of a precursor of prothromb Furthermore, when normal "silicone" plas and normal "glass" plasma were diluted 1

congenital hypoproconvertinemia plasma, ich provides an excess of every known sma factor except proconvertin, the differ- e between the two plasmas persisted.

Shaking with glass powder will partially rect the lengthened clotting time of dicu- rol plasma and produces an effect which proaches that of adding an equal amount serum to the dicumarol plasma. Moderate pression of proconvertin activity by dicumarol may be completely masked by shaking the plasma with glass powder. Such an exposure to a large glass surface area apparently accomplishes in a few minutes the same activation of proconvertin as has been shown (Alexander & Landwehr, 1949) to occur on permitting plasma to stand for several days in glass tubes. Similarly, the activation may be prevented by the addition of 0.2 to 0.5 unit of heparin per ml of plasma.

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Samuel I. Rapaport,
Knut Aas,
Paul A. Owren.

FIRST EUROPEAN CONGRESS ON CLINICAL CHEMISTRY

AMSTERDAM, SEPTEMBER 23—28, 1954

Preliminary notice.

The First European Congress on Clinical Chemistry will be held in Amsterdam from Thursday August 23rd until Tuesday 28th 1954 in the Royal Institute for the Tropics, 63, Mauritskade, Amsterdam-O.

The two leading subjects will be:

1. Isotopes in the Clinico-Chemical Laboratory.
2. The physical methods of measurement in connection with clinico-chemical problems.

These subjects will be presented in symposia, consisting of several lectures for which qualified speakers will be invited, and of free communications, related to the above subjects. For these communications a maximum of 15 minutes will be allowed.

Excursions to various institutes and laboratories and also to scenic parts of the Netherlands are being planned. Provision will be made by a special Ladies-Committee to entertain wives and guests accompanying the delegates.

Registration fees:

Full members per person: ƒ 25.— Neth. currency.

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Futher details, as to technical programm registration, methods of payment, variou classes of hotel-accommodation, excursion entertainment and receptions will be give by Dr. O. Meulemans, Secretary Genera Racinelaan 17, Utrecht, Netherlands.

INTERNATIONAL CONFERENCE ON THROMBOSIS AND EMBOLISI

An International Conference on Thron bosis and Embolism will take place in Bas from the 20th to 24th July 1954. The sul ject matter will deal principally with th aetiology, pathology, symptomatology, pr phylaxis and treatment of thromboembolism

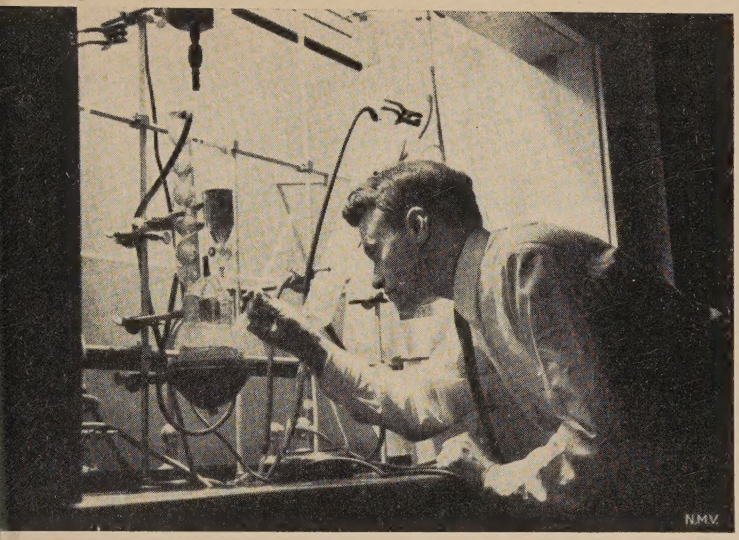
Further information may be obtained fro the Secretary General, Dr. W. Merz, Frauer spital, Basle.

CORRIGENDUM

Vol. V. No. 4, p. 373. Col. I, l. 9, f. b. the base effect, described later, is considere higher a.s.o.

Read: If the base effect, described late is considered, the △ a values in Table V will be still higher. When comparing g samples in the two reservoirs one shou always adjust the pressure to give the sam peak heights for m 28.

P. 374, col. II, l. 15 f.a.: measuremen like those given in Table IX. Read: Tab VIII.



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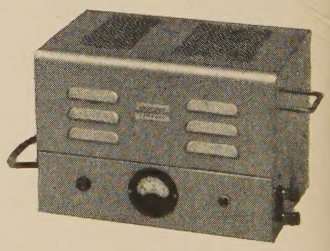

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All figures and illustrations are to be submitted in such form as to permit photographic reproduction ithout retouching or redrawing. Line drawings should be carefully drafted with black India ink on hite drawing paper or faintly bluelined paper.

The introduction should contain a clear statement of the purpose of the investigation and its elation to other work in the same field, but extensive reviews must be avoided.

The experimental procedure must be clearly described. However, methods should be given in xtenso only if they represent a new approach.

Every paper should end with a brief summary. References to the literature should be listed lphabetically at the end of the paper, and the names of the authors should be mentioned in the text with ne year of publication in brackets. The bibliography should conform to the style used in this journal: he author's name, initials, title of paper, abbreviation of journal, volume, page and year; in the case f books, the title in full, with the place and year of publication, and page.

No paper should exceed 12 printed pages. — Short reports of important findings, occupying not ore than 2 printed pages, will be accepted for prompt publication in the form of *Letters to the Editors.*

CONTENTS

B. HOOD, G. ANGERWALL, B. ISAKSSON AND G. WELIN: Studies on heparin and the lipemia clearing factor I 1

E. M. BLEGEN, H. N. HAUGEN AND K. ØRNING: Does intermittency of glomerular activity occur in humans? 8

A. TELKKÄ, S. WAHLSTRÖM AND R. KOULUMIES: Eosinophil cells in fish tapeworm (diphyllobotrium latum) carriers 15

H. N. HAUGEN: Glucose and acetone as sources of error in plasma "Creatinine" determinations 17

C.-B. LAURELL: Preliminary data on the composition and certain properties of human chylomicrons 22

Continued on cover IV

Mikroskoper og Medisinske Apparater.
Glassvarer for Laboratorier.

Jean Mette a/s

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CONTENTS *(Continued from cover III)*

G. ØSTLING AND G. TÖTTERMAN: Extrinsic (P^{32}) / intrinsic (P^{31}) phosphorus clearance ratio in man 25
V. JÄÄSKELÄINEN AND R. MÄKITALO: The reliability of the Scholander-Roughton method of microdetermination of the oxygen content of blood, using aerosol and saponin as the hemolytic agents 28
W. J. KAIPAINEN AND G. TÖTTERMAN: On the vitamin B_{12} content in feces in cases of pernicious tape-worm anemia 33
W. T. L. OHLSSON AND O. M. WILANDER: BAL-therapy in transfusion hemosiderosis 36
P. LOUS AND O. SYLVEST: A comparison of three methods, utilizing different principles, for the determination of uric acid in biological fluids 40
N. TYGSTRUP, K. WINKLER, E. LUND AND H. C. ENGELL: A clinical method for determination of plasma galactose in tolerance tests 43
L. ERIKSEN: A simple method for the preparation of protoporphyrin from washed erythrocytes 49
E. VARNAUSKAS AND L. WERKØ: Temporary occlusion of interatrial septal defect in man 51
U. S. VON EULER, S. HELLNER AND A. PURKHOLD Excretion of noradrenaline in urine in hypertension 54
V. FÜRST jr. AND R. LANGE: Evaluation of an acetic acid — sulphuric acid method for quantitative determination of cholesterol in serum 60
B. FRIIS-HANSEN: The measurement of deuterium oxide at low concentrations by the gradient tube method 65
L. A. CARLSON AND B. OLHAGEN: The electrophoretic mobility of chylomicrons in a case of essential hyperlipemia 70

Technique and practical problems:

A. DETTKER AND H. ANDURÉN: A new time-saving apparatus for paper electrophoresis 74
M. U. TSAO: A power source for filter paper electrophoresis 76

Letters to the editors:

M. E. PITKÄNEN: Detection of adrenaline and noradrenaline on paper chromatograms 78
T. HEIKEL, L. BÄCKSTRÖM AND M. HAKALA: Paper chromatography of uroerythrin 79
E. KVAMME AND L. HELLMAN: Isolation of pyruvic and alphaketoglutaric acids from blood and tissues in the presence of C^{14}-acetate 80
S. I. RAPAPORT, K. AAS AND P. A. OWREN: The coagulant activity of Russel viper venom 81
S. I. RAPAPORT, K. AAS AND P. A. OWREN: The increased coaguability of plasma exposed to glass 82

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